University of Alberta

The Pathogenesis of Human Immunodeficiency Virus in a Cohort of Patients Co-Infected With Hepatitis C Virus.

By

Samantha L. Bowker ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

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DEDICATION

I dedicate this thesis to my immediate and extended family, Neil, Val, Sarah, Robb, Jovan Bozinovski, and Joseph Parrottino, for their continual encouragement and support through the duration of this project.

ABSTRACT

The purpose of this study was to establish whether patients who are co-infected with HIV and HCV have a more severe disease progression of HIV as compared to patients who are mono-infected with HIV. This study is a retrospective cohort study that used an existing database of 1,276 HIV positive patients, 564 of whom have been tested for HCV. There were 238 HCV negative and 326 HCV positive patients.

Descriptive analyses were performed, and Kaplan-Meier (KM) survival analyses were estimated relating four selected HIV endpoints to HCV status.

The HIV endpoint CD4 count ≤100 was selected for the Cox analyses. HCV entered as the single main effect yielded a hazard ratio of 1.4, which was not statistically significant (p-value=0.51). The final Cox model chosen to be most predictive of reaching the HIV endpoint of CD4 count ≤100 included the variables HCV status, risk behaviour, effective HIV treatment, and AIDS-defining illness.

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LIST OF ABBREVIATIONS

ALT	Alanine Transaminase
AST	Aspartate Transaminase
AZT	Zidovudine
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention, Atlanta
CMV	Cytomegalovirus
DNA	Deoxyribonucleic Acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbant Assay
HAART	Highly Active Anti-Retroviral Therapy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IFN-α	Interferon Alpha
KM	Kaplan-Meier
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
PI	Protease Inhibitor
RIBA	Recombinant Immuno-Blot Assay
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
STD	Sexually Transmitted Disease
TB	Tuberculosis
UAH	University of Alberta Hospitals

CHAPTER ONE

HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND HEPATITIS C VIRUS (HCV) – AN INTRODUCTION

The purpose of this study is to establish whether patients who are co-infected with Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) have a more severe disease progression of HIV as compared to patients who are solely infected with HIV. This chapter consists of an overview and introduction to the epidemiology and pathogenesis of both HIV and HCV.

1.1 Introduction

The main focus of this study is on the health outcomes associated with co-infection of Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV). The intent is to ascertain whether HIV disease progression is different in patients co-infected with HCV. There will also be a smaller focus on the Hepatitis B Virus (HBV) and its interactions with HIV and HCV.

HIV infection can progress to Acquired Immunodeficiency Syndrome (AIDS), followed by death. In the process of HIV disease progression, opportunistic infections set in as CD4 T-lymphocytes are depleted, until they reach a critical value. The advent of the Highly Active Antiretroviral Therapy (HAART) which began to be available to patients in Edmonton in late 1996 has significantly improved outcome in HIV patients. It remains to be elucidated whether this fairly recent therapy will improve the outcome of HIV disease in HCV co-infected patients. HAART can delay or even prevent the progression of HIV disease; and thus, co-infected patients could ultimately die of other conditions, including liver failure or cirrhosis.

1.2 Epidemiology of HIV Infection

Prevalence and incidence are the most common measures of disease frequency in a population. The prevalence provides an estimate of the proportion of existing cases of a disease in a population at a point in time; therefore, it can be considered as the status of the disease in a population at a point in time. This measure of disease frequency can be compared to the incidence, which quantifies the number of new cases of a disease that develop in a population at risk during a given time period.

The provinces of British Columbia, Alberta, Ontario, and Quebec account for 85% of the population of Canada, and 95% of HIV and AIDS diagnoses in Canada. It is estimated that at the end of 1996, there were 40,100 people living with HIV in Canada (including those living with AIDS), and 49,800 at the end of 1999 [1]. This represents an increase of 24%. This prevalence can be broken down by risk category. Men who have had sex with men (MSM) occupied the largest proportion, comprising 25,300 or 63.1% of the prevalent 1996 infections. By 1999, MSM had 29,600 or 59.4% of the prevalent HIV and AIDS infections. Men who have sex with men include bisexual as well as homosexual men. The second largest group was injection drug users (IDUs) with 7,100 or 17.7% in 1996 and 9,700 or 19.5% in 1999, followed by heterosexuals with 5,500 or 13.7% in 1996 and 8,000 or 16.1% in 1999. Men who have sex with men and are also injection drug users (MSM-IDU) had a smaller proportion of prevalent HIV and AIDS infections with 1,700 or 4.2% in 1996 and 2,100 or 4.2% in 1999. Recipients of blood or clotting factor, perinatal transmission, and occupational transmission ("Other" risk category in Table 1.1) occupy the smallest proportion of prevalent HIV and AIDS infections with 500 or 1.3% in 1996 and 400 or 0.8% in 1999 [1]. Table 1.1 illustrates these numbers. It is important to note that many of the individuals that are living with HIV were infected several years previously.

There was an estimated 4,200 new HIV infections in Canada, in 1996 and 4,190 in 1999 [1]. While the estimated incidence in 1999 was essentially unchanged from 1996, the risk category distribution changed significantly. When broken down by risk category, the most common risk category for incident HIV infections in 1996 was among IDUs with

1,970 or 46.9%. However, the most common risk category for incident infections in 1999 was among MSM, with 1,610 or 38.4%. There was a 30% increase in the number of incident HIV infections per year among MSM (from 1,240 to 1,610) and a 27% decline in the number of new HIV infections among IDUs (from 1,970 to 1,430). There were no reported incident cases of HIV via occupational exposure, perinatal transmission, or among people who were recipients of blood or clotting factor ("Other" risk category in Table 1.2) [1].

It is apparent from Tables 1.1 and 1.2 that there is a remarkable difference in risk category distributions between prevalent and incident infections of HIV in Canada. The majority of prevalent HIV infections in Canada in 1996 were among MSM (63.1%), whereas IDU (46.9%) represented the most common risk for incident HIV infection in Canada, in 1996. However, in 1999 the majority of prevalent and incident HIV infections in Canada were among MSM with 59.4% and 38.4%, respectively. Since the early 1980s, when the HIV epidemic began in Canada, there has been a steady decline in the proportion of MSM who become newly infected with HIV, from over 80% during 1981-1983 to 29.5% in 1996, followed by an increase once again in 1999 to 38.4%. In contrast, there has been a remarkable increase in the proportion of IDU becoming newly infected with HIV, from less than 10% before 1986 to 24% in 1987-1990 and to 46.9% in 1996 [1]. However, there has been a recent decline in the proportion of newly infected IDUs to 34.1% in 1999. Thus, a large proportion of new HIV infections in Canada seems to occur among IDUs, explaining the substantial and growing importance of HIV and Hepatitis C co-infection.

Finally, there has also been a rise in the number of prevalent and incident HIV infections among heterosexuals. The incidence of HIV infections among heterosexuals has increased steadily in the last two decades to 21% of the incident HIV infections in Canada at the end of 1999. At the end of 1996, there were an estimated 4,600 women living with HIV in Canada. In 1996, women comprised 22.6% of incident cases of HIV infection in Canada, whereas in 1986, women comprised less than 10% of incident cases of HIV in Canada. This demonstrates a considerable increase in the proportion of women contracting HIV in Canada [1].

Therefore, in addition to the recent increase in the proportion of newly infected MSM, the increase in HIV infections among women indicates there is also a shift in the HIV epidemic towards heterosexuals (especially women). Clearly, there is a changing face of the HIV epidemic in Canada, illustrating the urgency to focus on specific risk groups, such as MSM, IDUs, and women.

1.3 Pathogenesis of HIV Infection

The course of HIV infection generally starts with a prolonged asymptomatic phase. Some patients exhibit a brief, one- to two-week, self-limited illness within a few weeks of acquiring HIV infection. This is followed by a prolonged asymptomatic phase consistent with a persistent virus infection. This symptom-free phase, also known as the clinical latency period, varies from person to person, but lasts an average of about 10 years for patients receiving antiretroviral therapy. Studies of HIV antibody seropositive cohorts have shown that group means of CD4 cell counts and CD4:CD8 ratios gradually decrease over time, and that declines in CD4 cell counts to very low levels (below 200 cells/mm^3) were predictors of Acquired Immunodeficiency Syndrome (AIDS) [2, 3]. An increasing CD4 percent was found to have a significant protective effect, reducing the risk of progression to AIDS [4]. However, a sustained increase in CD4% only occurs with antiretroviral treatment. CD4 cell count and CD4% are now routinely used in monitoring the progress of HIV-antibody positive individuals. The Centers for Disease Control and Prevention (CDC) has added a CD4 cell count of $\leq$200 cells/mm^3 to the clinical case definition of AIDS [5]. The study by Yarchoan et al. assesses CD4 cell count and the risk of death in HIV-antibody positive patients receiving antiretroviral therapy in the era of single drug treatment [6]. For the patients who died, all but one had a CD4 cell count that fell below 50 CD4 cells/mm^3 (p-value < 0.001), and the mean of the last three CD4 counts obtained before death was 7.7 CD4 cells/mm^3. The median survival of these patients, once their CD4 cell counts fell below 50 CD4 cells/mm^3, was 12.1 months.

Therefore, a CD4 count of 50 cells/mm^3 is associated with a high, short- to medium-term mortality in the absence of effective antiretroviral therapy.

There are several variables that may affect the progression of HIV to AIDS. Older age at the time of infection with HIV is known to be associated with a shorter time to the development of AIDS [7]. In the study by Darby et al 1996, it was assessed whether the effect of age at seroconversion was from confounding by time since seroconversion, calendar year, or haemophilia type or severity by calculating the excess death rate associated with HIV infection after adjustment for these factors. The adjustment slightly increased the effect of age at seroconversion. Furthermore, mortality from conditions associated with Hepatitis C infection had no effect on age-related differences in survival. Survival is therefore strongly correlated with age at seroconversion, with 86% of patients who seroconverted before the age of 15 surviving for 10 years after infection, compared with only 12% of patients who seroconverted at or after age 55, and with a smooth gradient in between [7].

Viral load (HIV RNA level) is another strong determinant of HIV progression [8, 9]. Viral load is a measurement of the number of viral particles in plasma. A lower HIV RNA is associated with a more favourable prognosis or outcome in HIV-infected individuals. However, viral load testing has only been available since late 1996. Initially, the lowest limit of detection for these tests was 500 RNA copies/ml. This test has become more sensitive, and the current lowest limit of detection is 50 RNA copies/ml. For the purposes of this study, it was established that an undetectable viral load is considered to be <50 RNA copies/ml, and two consecutive undetectable viral loads indicate an effective treatment regimen in the patient. The treatment regimens for HIV differ somewhat, but HAART or a combination of antiretovirals (ARV) are considered to be the current preferred treatment regimen. These therapies, which combine reverse transcriptase and protease inhibitors or non-nucleoside reverse transcriptase inhibitors, modify the prognosis for HIV infection with a dramatic improvement in viral load, a variable but usually positive change in immune status, and a marked improvement in prognosis [10]. This improvement in immune status is achieved by a decrease in HIV viraemia and an increase in CD4 and CD8 cell counts.

1.4 Epidemiology of HCV Infection

Hepatitis C Virus was first cloned in December 1989, and is the cause of 90% of parenterally-acquired non-A, non-B Hepatitis [11]. Prior to this time, there was no test readily available to detect Hepatitis C antibody in the population. Although a very small percentage of patients with histories of sexual exposure (1-10%), household exposure (1-10%), occupational exposures (1-2%), or hemodialysis (20%) have become infected with HCV, the highest rates of transmission are via parenteral routes [11, 12, 13]. Thus, transfusion of blood or blood products, transplantation of organs from infected donors, and sharing of contaminated needles among IDUs are the most efficient means for transmitting HCV. Up to 90% of IDUs are positive for antibodies to HCV [14, 15]. Furthermore, the prevalence of HCV infection was close to 100% in hemophiliac patients who received pooled blood products before HCV screening became available [15, 16, 17, 18]. Currently, however, all blood donors in Canada are screened for anti-HCV, thus virtually eliminating the possibility of infection via this route; the current risk of transfusion-associated HCV infection is estimated at 0.01%-0.001% per unit transfused [19]. IDU now represents that risk behaviour associated with the great majority of new infections with HCV mono-infection and with HIV and HCV co-infection. Acquisition of Hepatitis C Virus infection is very rapid among new IDUs with 50-80% infected within 6-12 months [18, 20].

National reporting of HCV infection in Canada started in 1992. IDU is associated with at least half of HCV infections, but this likely is an underestimate of the true number [21]. The receipt of blood and blood components, especially before 1990, is the second most common risk factor for HCV infection. However, this risk has decreased markedly from approximately 30% in the 1960s to 1.3% in the late 1980s, to 1 in 103,000 today. The number of cases reported has increased dramatically from 1992 (1,321 cases) to 1997 (19,571 cases), mainly because of increased recognition of previously acquired infection [1]. The overall prevalence of Hepatitis C in Canada is 0.8%; therefore, an estimated

240,000 individuals may be infected [1, 22]. In British Columbia the prevalence is 1.4%, and in Newfoundland it is 0.08% [23]. No other figures on prevalence were given for the other provinces and territories.

1.5 Pathogenesis of HCV Infection

The natural course of HCV infection is poorly understood primarily because of the prolonged course of the disease (another persistent virus infection), the small number of patients in whom the date of infection is known, and the relatively recent availability of a reliable diagnostic test. The first generation ELISA test used to detect anti-HCV was licensed in 1990; a more sensitive, second generation ELISA test was licensed in 1992. However, this more sensitive second generation ELISA test was not widely used at the Provincial Lab at the University of Alberta Hospital until late 1992 or early 1993. Furthermore, the pathophysiology of Hepatitis C Virus is still largely debated, especially the respective contributions of direct viral cytopathic and immune-mediated effects on liver damage [24]. The disease progression of Hepatitis C Virus is known to have a variable course, which may be exacerbated by a number of different factors, such as age, gender, alcohol consumption, virus characteristics (genotype), and co-infection with other viruses such as HIV and Hepatitis B Virus (HBV). Therefore, the range of disease states following Hepatitis C Virus infection is quite broad [20].

When an individual becomes infected with Hepatitis C Virus, the newly acquired infection is referred to as acute Hepatitis C. Acute Hepatitis C infection is usually asymptomatic, or the symptoms are so non-specific that the onset of infection is often unrecognized. Only 25-35% of patients will develop malaise, weakness, or anorexia [20]. However, virtually all patients develop liver cell injury, which, at this stage of infection, may be characterized by elevated serum alanine aminotransferase (ALT) levels. Hepatitis C Virus is not easily cleared by the host's immunologic defenses; thus, a persistent or chronic form of the infection will develop in as many as 85% of patients infected with Hepatitis C [11, 20, 25, 26, 27]. Spontaneous recovery from Hepatitis C occurs in a minority of patients and is characterized by disappearance of HCV RNA from serum and a return of liver enzymes (ALT) to normal.

Those who develop chronic infection have persistent viraemia and HCV RNA levels that remain detectable in serum indefinitely. Antibodies to HCV (anti-HCV) and HCV RNA can be found in virtually all patients. In addition, most individuals will have persistently or intermittently elevated concentrations of serum aminotransferases; however, the correlation between severity of liver injury and degree of raised aminotransferase activity in an individual patient is poor [27]. In fact, approximately one-third of chronic Hepatitis C patients will have persistently normal ALT levels; nevertheless, these patients may exhibit a marked degree of chronic hepatitis, as indicated by histologic evidence [18, 20]. A decrease in albumin levels is correlated mainly with advanced liver damage or cirrhosis, and so tends to be informative only after it is too late. Chronic HCV infection is insidious and has a slow rate of progression in the majority of patients during the first two decades after infection, and usually runs an asymptomatic clinical course [28]. Less than 20% of patients may experience nonspecific, vague symptoms such as fatigue and malaise, and often these symptoms do not present until the patient begins developing advanced liver disease [20, 27].

In chronic Hepatitis C, liver cell necrosis occurs. As the disease progresses, the inflammation and liver cell death may lead to fibrosis [20]. The extent of fibrosis in liver tissue determines the stage of disease in a patient and is an important predictor of the development of cirrhosis [27, 29]. Mild fibrosis is confined to the portal tracts and immediately adjacent parenchyma, whereas more severe fibrosis leads to bridging between portal tracts and between portal tracts and veins [20]. The more severe form of fibrosis is often referred to as a state of diffuse fibrosis, which may progress to cirrhosis. The most common histological pattern of chronic HCV corresponds to mild fibrosis. It is believed that approximately 20% of chronic hepatitis C leads to cirrhosis within 2-3 decades of the onset of infection [12, 16, 18, 20, 27, 30], although there tends to be considerable variation in disease outcome. There is a wide range of susceptibility to the development of cirrhosis, but it seems more frequent in males, those infected after the age of 50 years, in patients who consume substantial amounts of alcohol, and in patients who are immunocompromised (HIV) [31]. In one study, the cumulative progression rates to cirrhosis were 8.4%, 22.6%, and 31.3% at the end of 5, 10, and 15 years of follow-up, respectively [29]. HCV-associated end-stage liver disease is the most frequent indication for liver transplantation among adults [18]. Liver transplantation is the only means of restoring health to patients with end-stage liver disease from HCV.

Once a patient has cirrhosis, several complications secondary to liver failure and/or portal hypertension may develop, such as jaundice, ascites, variceal hemorrhage, and encephalopathy. The development of any of these complications marks the transition from a compensated to a decompensated cirrhosis [20]. As mentioned before, most patients who develop cirrhosis will have detectable ALT elevations. However, the inconsistent relationship between ALT levels and disease severity, and the absence of any more reliable serological marker of disease activity, indicates that not only should a single ALT measurement be considered, but rather the pattern of ALT levels over time. Furthermore, histology remains the only reliable indicator of liver injury.

There is an increased risk of developing hepatocellular carcinoma in patients chronically infected with Hepatitis C; however, virtually all cases of chronic Hepatitis C-related hepatocellular carcinoma occur once cirrhosis has been established. Hepatocellular carcinoma may develop in as many as 1% to 4% per year of patients with established cirrhosis, and it has been estimated that 1% to 4% of patients infected with HCV may go on to develop hepatocellular carcinoma [18, 27]. Among patients with cirrhosis from Hepatitis C, hepatocellular carcinoma develops more commonly in men and in older patients [20]. The study by Ikeda et al 1998 found cumulative progression rates to hepatocellular carcinoma of 4.8%, 13.6%, and 26.0% at the end of 5, 10, and 15 years, respectively in patients with documented cirrhosis [29]. These Japanese rates of hepatocellular carcinoma are extraordinarily high, however, and quite inconsistent with what is seen in Canada [Stan Houston, personal communication]. Although high rates of hepatocellular carcinoma after HCV infection have been recorded, the time-course and cumulative mortality risk have not been documented. Similarly, although persistent HCV infection causes chronic liver disease, there is little quantitative information about its effect on mortality [17]. However, the study by Ikeda et al 1998 has documented survival rates of 99.0%, 97.4%, and 90.5% at the end of 5, 10, and 15 years of follow-up following diagnosis of Hepatitis C, respectively [29].

There are extrahepatic manifestations that may develop as a result of chronic Hepatitis C Virus infection. These are of immunologic mechanism origin, and they include glomerulonephritis, essential mixed cryoglobulinemia, arthritis, B cell non-Hodgkin's lymphoma, porphyria cutanea tarda, and thyroiditis [20, 27, 32]. The role of HCV in the pathogenesis of most of these conditions remains obscure.

Disease progression to cirrhosis and liver disease has not been conclusively linked to the mode of acquisition of Hepatitis C or to particular risk groups [20]. There are a limited number of studies that have investigated disease progression by source of infection. In a study by Darby et al. 1997, mortality from liver disease was 16.7 times higher than that in the general population, and 5.6 times higher for liver cancer among patients infected with Hepatitis C Virus [17].

Several studies report a strong effect of age at exposure. A more rapid disease progression has been found in older individuals infected with HCV [17, 28, 31, 33]. In addition, post-transfusion chronic HCV has been shown to have more severe histological changes than does chronic HCV from parenteral drug use [34]. Similar findings in the study by Delladetsima et al. revealed that there was a relatively high frequency of minimal chronic HCV and a statistically lower incidence of cirrhosis and therefore of liver cancer in intravenous drug users. Furthermore, Ikeda et al 1998 found a history of blood transfusion to be independently associated with disease progression to cirrhosis and hepatocellular carcinoma [29]. The phenomenon of a milder histological pattern in intravenous drug users perhaps can be ascribed to the younger age of these patients as compared to that of the post-transfusion patients. The larger initial inoculum associated with blood transfusion and the finding of higher levels of viral replication in these patients adds support to these findings. According to a study by Datz et al 1999, widely different patterns of disease progression occur in a group of patients, all infected with the same virus strain at the same time even though they all had similar epidemiological backgrounds. Therefore, they concluded that host factors must play a critical role in determining the natural history of disease progression for each individual subsequent to Hepatitis C Virus infection [30].

In a multivariate analysis, Bellentani et al 1999 showed that, independent of age, sex, and alcohol intake, genotype 1b infection is a major risk factor associated with the presence of cirrhosis and/or hepatocellular carcinoma [35]. In Canada, the most common genotype is genotype 1, accounting for perhaps two-thirds of cases. When 257 Hepatitis C Virus positive patients were typed in Kelowna, 48%, 19%, 6%, 3%, and 22% were found to be of genotypes 1a, 1b, 2a, 2b, and 3a, respectively [23]. Despite the initial suggestions that infection with genotype 1b is more likely to lead to cirrhosis or hepatocellular carcinoma, no clear association with disease outcome or severity has been demonstrated; it remains to be demonstrated whether genotypes have measurable clinical significance except in their effect on treatment efficacy [27].

It is not yet known why some patients have the ability to naturally clear the virus from their bloodstream during the acute period of infection. In addition there are still a number of questions as to why disease severity varies from person to person. The diversity of patients with respect to age, gender, presence or absence of cirrhosis, type of cirrhosis, alcohol consumption, method of acquisition of HCV, and evidence of past or present co-infection with other viruses such as HBV or HIV emphasizes the complex, multifactorial and probably interactive relationships involved in the natural history or pathogenesis of Hepatitis C [29].

1.6 Management of HIV and HCV

There is no vaccine currently available for either HIV or HCV and unfortunately, while the drugs that are available do not provide a cure, they are a means of slowing down the progression of disease and improving both the quality and quantity of life in patients afflicted with these infections. There are different drug regimens for patients mono-infected with HIV and mono-infected with HCV.

1.6.1 HIV Management

Treatment for HIV has improved substantially in the past 5 years with the advent of highly active anti-retroviral therapies (HAART). The goal of HIV treatment is to decrease the viral load and increase the CD4 cell count, thus stopping the progression of HIV disease in patients who respond to the treatment. Currently, there are three different groups of antiretroviral drugs available; these are used in combination in HAART therapy: 1) non-nucleoside reverse transcriptase inhibitors (NNRTIs), 2) nucleoside reverse transcriptase inhibitors (NRTIs), and 3) protease inhibitors (PIs). Drugs in the NRTI group comprise several of the older anti-HIV medications. The most familiar example is the drug AZT (the generic name is Zidovudine). The majority of patients with clinical or laboratory evidence of advanced disease will begin therapy as soon as possible. The current practice is to offer therapy to asymptomatic patients depending on their viral load and CD4 cell count results, as well as patient preference, and ability to adhere to the therapy. The first treatment that physicians often prescribe is a combination of three drugs, usually two NRTI drugs, and either a protease inhibitor or a NNRTI. These reverse transcriptase inhibitors inhibit the action of reverse transcriptase in cells, thereby blocking an early step in the HIV replication process. The NNRTI drugs are a newer group of anti-HIV drugs, which must be used in combination with other anti-HIV drugs to remain effective. Similar to the NRTIs, NNRTIs also block the action of reverse transcriptase in cells, although they are clinically quite different. Protease inhibitors are the most potent anti-HIV drugs currently available and are considered to be a critical advance in managing HIV. The PIs are often prescribed in combination with NRTIs to enhance their effectiveness. PIs target one of the last, but most vital stages of the HIV replication process, by preventing HIV from infecting additional CD4 cells.

Different HIV drugs attack the virus at different stages of development. Reverse transcriptase inhibitors start working soon after HIV infects a cell, whereas PIs work at a much later stage. In most studies, treatments that combine RTIs and a PI have proven to be more powerful than RTIs alone in slowing HIV reproduction and increasing the number of CD4 cells.

A fundamental practical issue surrounding the HIV drug regimen is compliance with a strict schedule. Medication must be taken with a very high degree of consistency at scheduled times. Patients that do not follow a strict drug regimen are at great risk of treatment failure and developing drug-resistance. Drug resistance occurs when a drug that was previously effective becomes less and less effective in fighting HIV. This occurs because HIV is characterized by an extraordinarily high rate of spontaneous mutations; drug treatment selects for mutations in the HIV virus that confer resistance to therapy with that drug. By adhering to the drug regimen, and therefore maintaining a very low viral load and a very small number of viral replication events, fewer mutations are produced, so resistance is less likely and the medication will presumably work longer. The use of several drugs together, each with a different genetic mechanisms of resistance markedly reduces the statistical possibility of a virus resistant to all the drugs. As a greater number of more powerful drugs emerge, the long-term outlook for HIV infected patients continues to improve.

1.6.2 HCV Management

Until recently, interferon alpha (INF-α) was the only therapy available for patients with chronic hepatitis C. INF-α is a potent immune modulating and antiviral compound, which exponentially reduces serum HCV-RNA levels in a rapid initial decrease, followed by a shallower decrease in serum Hepatitis C levels. However, in a study by Begemann et al. less than 30% of chronic hepatitis C patients showed a sustained virological response [26]. A sustained virological response of 30% is actually quite high; more often only 15-20% of chronic hepatitis C patients will show a sustained virological response. A sustained virological response is defined as persistently normal serum aminotransferase concentrations and the absence of HCV RNA from serum 6 months after treatment completion. In 345 chronic hepatitis C (CHC) patients relapsing after pretreatment with INF-α mono-therapy, a significant increase in sustained response was achieved with a combination of INF and ribavirin compared to patients treated with INF alone [26]. The low rate of sustained response with INF-α treatment alone is primarily the result of a high rate of relapse following discontinuation of treatment. HCV genotype, serum HCV viral load levels, and endogenous INF pretreatment levels appear to be predictive of response to treatment [26]. The study by Begemann et al. (1999) also indicates an improvement in outcome and long-term benefit with the combination therapy of INF-α and ribavirin. INF-α and ribavirin now represents the current standard of care for HCV.

It is believed that Hepatitis C can be cured; this conclusion is based on the long-term virologic remission which appears to be permanent around 35%-40% of the time depending on patient characteristics such as genotype and viral load levels. The majority of studies focusing on treatment options for chronic hepatitis C have not dealt with the issue of HIV and HCV co-infection; there thus is very limited information on the treatment of co-infected individuals. It is unclear whether the combination therapy of INF-α and ribavirin would be as effective in co-infected patients; this issue warrants further research.

Table 1.1: Prevalence of HIV Infection in Canada at the End of 1996.

Point estimates and uncertainty ranges for numbers of prevalent HIV infections in Canada at the end of 1999 compared with the point estimates for 1996, by risk category.

	MSM	IDU	Heterosexual	MSM-IDU	Other	Total
1999 Range	29,600 (26,000-33,400)	9,700 (8,100-11,800)	8,000 (6,300-10,100)	2,100 (1,700-2,600)	400 (330-470)	49,800 (45,000-54,600)
1996 Range	25,300 (22,500-28,100)	7,100 (6,000-8,500)	5,500 (4,400-6,900)	1,700 (1,400-2,100)	500 (430-580)	40,100 (37,100-43,300)

Source: Bureau of HIV/AIDS, STD and TB, LCDC, Health Canada, November 2000.

Table 1.2: Incident HIV Infections in Canada for 1996.

Point estimates and uncertainty ranges for numbers of incident HIV infections in Canada in 1999 compared with point estimates in 1996, by risk category.

	MSM	IDU	Heterosexual	MSM-IDU	Other	Total
1999 Range	1,610 (1,190-2,060)	1,430 (1,030-1,860)	880 (610-1,170)	270 (190-360)	0	4,190 (3,310-5,150)
1996 Range	1,240 (1,050-1,460)	1,970 (1,600-2,400)	700 (540-910)	290 (230-370)	0	4,200 (3,700-4,750)

Source: Bureau of HIV/AIDS, STD and TB, LCDC, Health Canada, November 2000.

CHAPTER TWO

CO-INFECTION OF HUMAN IMMUNODEFICIENCY VIRUS AND HEPATITIS C

This chapter provides a discussion of the literature with respect to HIV and HCV co-infection. A description of the pathogenesis of both HIV and HCV in co-infected individuals is also given.

2.1 Co-infection with HIV and HCV

Because HIV and HCV share the same route of parenteral transmission, co-infections with HIV and HCV are frequently found in IDUs, haemophiliacs who received multiple transfusions with clotting factor concentrates before 1985, and transfusion recipients prior to November, 1985 [12, 15, 36, 37]. In November 1985, testing for HIV became commercially available in Canada; however, it was not until 1990 that Hepatitis C testing became commercially available in Canada.

Several reports propose that HIV infection enhances HCV replication, either by HIV-induced immunosuppression [38, 39], or by a direct or indirect HIV-HCV interaction [14, 36]. There are many conflicting results with respect to the possible interactions between HCV and HIV. Several studies have found an increase in HCV RNA levels in patients who seroconverted to HIV [15, 25, 40, 41, 42]. However, the reasons why HCV viraemia is increased remain unclear. The study by Cribier et al. [15] assessed a cohort of patients composed primarily of intravenous drug users. They did not find a correlation between HCV viraemia and CD4 cell counts, p24 antigenaemia, ALT levels, or HIV viral load. Cribier et al. found HCV RNA levels were strongly increased in co-infected patients, but they concluded that Hepatitis C viral load was not correlated with the immunosuppression induced by HIV [15]. Therefore, their data did not support the hypothesis of a direct interaction between HIV and HCV; alternatively, they suggest that indirect factors present in HIV-infected patients may play a role in the enhancement of HCV replication. This study indicates that co-infected patients have increased levels of

HCV RNA, and that the antibody response towards HCV is impaired in the HIV-positive group [15]. These findings are in agreement with those of another study. Sherman et al. [41] did not find any correlation between HCV viraemia and the CD4 count.

Some studies of haemophiliac patients suggest that there is, in fact, a viral interaction between HIV and HCV because of the higher HCV viraemia in HIV-infected individuals [25, 37, 38, 40]. Telfer et al. was the first to hypothesize the direct interaction between HIV and HCV [38]. This idea was strengthened when Eyster et al. [40] found a strong negative correlation between HCV RNA levels and CD4 counts, which suggested that immunodeficiency may increase HCV replication. More specifically, there was a significant increase in HCV viral load after HIV seroconversion in haemophiliac patients who were initially infected with HCV. Although it is evident that there is, in fact, an association between HIV infection and increased HCV replication, the mechanism by which this occurs remains to be elucidated. The literature tends to indicate that this association is not mediated through HIV immune suppression. In the absence of a direct interaction between HIV and HCV, there must be another explanation for the increased HCV viraemia in co-infected patients. Yoshimura et al. [43] found an inverse correlation between the titre of antibody to Hepatitis C and the degree of Hepatitis C viraemia. It is suggested that the altered immunity to HCV may also be involved in the increase of HCV viraemia observed in co-infected patients.

Beld et al. [36] examined HIV and HIV-induced immunodeficiency enhancing HCV replication among HCV seroconverters. The study was a retrospective cohort that assessed the above in a population of injecting drug users. The major limitation of this study was the small sample size of 19 people. Virological (HCV and HIV RNA levels) and immunological (CD4, CD8 cell counts, and anti-CD3 reactivity) parameters were measured over a period of 1 to 9 years of follow-up. The objective of this study was to determine whether there was an association between the virological and immunological parameters. Throughout the study, no patients received antiviral therapy. Essentially, HCV RNA levels were higher among HIV antibody positive patients than in HIV antibody negative patients. HCV RNA levels also were higher among patients who seroconverted to HIV than among HIV negative people. When HCV RNA levels were compared before and after HIV seroconversion, levels of HCV RNA were indistinguishable between HIV seroconverters before seroconversion and HIV-antibody negative patients, as they were between HIV seroconverters after seroconversion and HIV-antibody positive patients.

One of the main findings was a statistically significant inverse association between CD4 cell counts and HCV RNA levels across the entire study population ($p<0.001$). This association remained significant when the HIV-antibody negative and positive populations were considered separately. The underlying mechanism behind this association remains to be elucidated. However, it is known that the presence of HIV infection causes a decline in CD4 cell counts over time, as well as an increase in HCV RNA levels over time in co-infected patients [14]. No association was established between HCV RNA levels and the duration of HCV infection, possibly owing to the shorter mean duration of follow-up (5.4 years). Thus, the conclusion was that HIV infection or immune suppression secondary to HIV leads to enhanced HCV replication.

A lack of association between CD4 cell counts and HCV RNA levels in some studies may be the result of a sampling bias of immunological parameters and HCV RNA levels, or a deficiency of measurements taken before and after seroconversion. In order to eliminate a sampling bias, the sampling of immunological parameters and HCV RNA levels should be representative of the entire cohort. Furthermore, it is crucial that the sampling method be reproducible.

There are many inconsistencies in the literature with respect to HIV and HCV co-infection. However, the consistent findings in the published studies agree that HCV viral load is increased in HIV positive patients, regardless whether it was an old or new HIV infection. Inconsistencies in the literature mainly concern the means by which the HCV viral load is increased in HIV patients. The findings by Telfer et al. [25, 38], Beld et al. [36], and Eyster et al. [37, 40] concluded that HCV viral load varies inversely with CD4 count, suggesting a direct viral interaction between HIV and HCV. In contrast, Sherman et al [41] and Cribier et al [15] did not find any correlation between HCV viraemia and CD4 cell count. These are the studies that tend to refute a direct interaction between the two viruses, and instead suggest indirect factors present in HIV patients may be responsible for enhancing HCV replication. There are yet other studies that believe HIV infection enhances HCV replication via HIV-induced immune suppression [38, 39]. Thus, it is evident that, while HCV viraemia is increased in HIV positive patients, the underlying mechanism(s) by which this association occurs remain to be elucidated.

2.2 Managing HIV and HCV Co-Infection

There are several reasons why treatment options for HIV and HCV co-infection must be considered. Firstly, HIV-infected patients currently have a longer survival rate owing to better management of HIV and its associated opportunistic infections. Co-infected patients should be concerned increasingly with the possibility that their HCV may adversely affect the progression of HIV because there is now evidence that co-infection may be associated with a more rapid evolution from HIV infection to AIDS [37, 44, 45, 46, 47]. Another concern is that the HIV is well controlled owing to the powerful HAART therapies, and co-infected patients die of cirrhosis or liver failure. Finally, patients co-infected with HIV and HCV are often the most challenging to treat. The major problem they face is compliance with a daily drug regimen. The behavioural issues surrounding many co-infected patients prevent them from adhering to a daily schedule. A large majority of the co-infected patients are intravenous drug users, and they live in sub-optimal conditions, many of them on the streets of the inner city. Because individuals with chaotic lifestyles find it difficult to meet the demands of strict adherence to a drug regimen, they are also faced with the potential issue of HIV drug resistance (personal communication, Dr. Stan Houston).

The natural course of chronic Hepatitis C is altered by underlying HIV infection in terms of rapid progression to liver disease [26]. An explanation for this may be that the reconstitution of the immune system following HAART might be accompanied by an increase in CD4 and CD8 cells, thus causing hepatic inflammation [26]. This worse prognosis of liver disease, however, is not limited to patients receiving HAART therapy.

An abstract by De Sanctis et al. reports INF-α showing a statistically significant long-term benefit on liver disease from chronic viral hepatitis in patients with HIV infection, albeit no significant increases in CD4 counts were observed [48]. This finding is consistent with a study by Del Pozo et al. 1998 [33]; INF-α was useful in treating chronic Hepatitis C in HIV-infected individuals and the complete response of 57.4% was similar to the one observed in non-HIV patients. However, patients with CD4 cell counts $\leq 200/\text{mm}^3$ had a poor response to treatment with INF-α, and a significant increase in the absolute number of CD4 cells was observed in patients with a complete response. The 26% non-response rate in this study could also have been the result of other factors, namely viral load and HCV genotype, which may influence the efficacy of treatment.

ZDV is a component of HAART, which targets HIV; it has no effect on HCV. The life expectancy in HIV mono-infected patients has significantly increased because of HAART; however, this is balanced by a more pronounced risk of liver mortality in HIV positive patients with chronic hepatitis C [26]. The response to INF-α therapy in co-infected patients has been found to depend largely on the underlying immune deficiency; Mauss et al. concluded that INF-α is indicated in patients with a CD4 cell count >300 cells/ml [8].

2.3 Literature that Assesses Co-Infection with HIV and HCV

The literature on HIV and HCV co-infection either assesses the disease progression of HIV in co-infected individuals or the disease progression of HCV in co-infected individuals. There is significantly more literature published on the progression of HCV in co-infected individuals.

2.3.1 The Pathogenesis of HCV in Co-Infected People

An abstract by Andreu et al. describes how HIV and HCV co-infection modified the natural history of chronic parenterally-acquired Hepatitis C with an unusually rapid progression to cirrhosis [49]. This finding is in agreement with several other studies [17, 25, 26, 37, 50, 51, 52]. The mean time from acquisition of HCV infection to cirrhosis was significantly longer in HIV negative patients than in co-infected patients ($p<0.001$). In addition, HCV viral load was almost 10 times higher in the HIV positive group, but these differences did not attain statistical significance. This study was primarily limited by its small sample size (n=20), and this may be one reason that a statistically significant difference was not found between these two groups. Additional studies are needed to elucidate the role, if any, that the HCV viral load may have on the severity and outcome of liver damage in co-infected people.

Bierhoff et al. 1997 assessed the influence of HIV co-infection on preexisting long-term chronic Hepatitis C. HIV co-infection was found to accelerate the course of preceding long-term chronic Hepatitis C with a more marked liver fibrosis. A study by Eyster et al. found similar results; their principal finding was that HIV positive haemophiliacs have more aggressive HCV chronic liver disease compared to HIV-negative patients. Several other authors [25, 50] have confirmed this finding. Moreover, Darby et al. 1997 suggest that co-infection with HCV and HIV leads to higher mortality from liver-related diseases than infection with HCV alone [17].

Specifically, in one study, HIV co-infection was found to accelerate the progression of hepatic decompensation, particularly in patients with a rapid rate of decline of CD4+ cell counts and p24 antigenaemia [25]. The authors concluded that HIV co-infected patients, particularly haemophiliacs, are threatened by a higher rate of post-hepatitic cirrhosis (46.2%) and cholestatic hepatopathy. None of the patients with HCV mono-infection died with liver cirrhosis. The more pronounced liver fibrosis in co-infected patients is of particular interest because this pattern of fibrosis is unusual in chronic HCV. This suggests an independent fibrogenic effect of HIV infection or a synergistic effect of HIV and Hepatitis C. In addition, cholestasis is a very rare finding in Hepatitis C. Thus, in this study, the course of liver disease in co-infected patients is mainly determined by cirrhosis caused by hepatitis C and by HCV-associated cholestatic hepatopathy at later stages of immunodeficiency.

The results from a study by Zylberberg et al. 1998, show that tritherapy for HIV infection does not modify replication of Hepatitis C Virus in co-infected patients; neither the biological activity of HCV-related chronic Hepatitis nor the Hepatitis C viral load were modified despite immune restoration (i.e. a significant increase in the CD4 and CD8 cell counts, and a decrease in the HIV RNA load) [10]. Another study by Zylberberg et al. 1998, describes an unusual case of rapidly evolving HCV-related cirrhosis that paralleled restoration of immune status in an HIV-infected patient. This case suggests, but does not definitively conclude, that there is a link between protease inhibitor-related immune restoration and hepatic deterioration, since a twofold higher incidence of cirrhosis and a rapidly evolving course of HCV-related hepatitis have been described for HCV-HIV co-infected patients [50, 53]. The findings by Zylberberg et al. 1998 indicate that a dramatic improvement in immune status in HIV-infected patients may have a deleterious impact on the course of HCV co-infection. A similar phenomenon has been observed with other opportunistic infections, such as Mycobacterium avium, Cytomegalovirus (CMV), and Tuberculosis (TB) [Dr. Stan Houston, personal communication].

2.3.2 The Pathogenesis of HIV in Co-Infected People

There are potential concerns with respect to HIV disease progression in co-infected patients. HIV mono-infected patients tend to differ temporally from co-infected patients in Western Canada, because the epidemic shifted to IDUs only 6-7 years ago. The issue of HIV and HCV co-infection is fairly recent, emerging after December 1989, whereas HIV has been recognized as a problem for approximately 16 years (since 1984-85) and AIDS for 19 years (since 1981). Moreover, HIV mono-infected patients differ in behaviour from co-infected patients. HIV patients are more often homosexual or bisexual, with strong support networks and good living conditions. On the other hand, co-infected patients are more likely to be aboriginal in Northern Alberta, poor, and live very chaotic lifestyles in association with IDU. Finally, since late 1996, HIV treatment may have a very powerful effect on the outcome of HIV disease progression. In fact, HIV treatment may overwhelm any impact of HCV on HIV disease progression.

According to a French study by Chaillou et al. [54], HCV positivity in AIDS patients does not influence survival. HCV positivity was not a prognostic factor for AIDS survival compared with people who were HCV negative. These results were confirmed in several other studies. The study by Dorrucci et al. [55] compared the progression rates to AIDS for individuals who were co-infected with HCV and for those infected with only HIV. Progression rates to AIDS were similar for HIV-seropositive persons who were co-infected with HCV and those who were not co-infected. Furthermore, two cross-sectional studies suggested that co-infection with HCV does not influence clinical or immunological progression of HIV disease [56, 57]. Staples et al. [58] found no deleterious effect from HCV co-infection on survival in a group of 350 HIV-infected (115 HCV-positive and 235 HCV-negative) patients. These patients were part of a cohort of >1800 patients followed at the Atlanta Veterans Affairs Medical Center.

There are studies, however, that have found a negative impact of HCV and HIV co-infection on HIV disease progression. One study found co-infection of HCV and HIV to be associated with a more rapid evolution to AIDS [37]. In the study by Ockenga et al [44], co-infection was associated with reduced survival in patients with AIDS compared to controls. Another study evaluated a cohort of 111 haemophiliac patients. Patients infected with Hepatitis C genotype 1 experienced a significantly more rapid progression to both AIDS (p=0.009) and death (p=0.007) than did those infected with other genotypes [46]. Piroth et al [45] followed a cohort of 238 HIV positive patients from 1993-1996, 119 of whom were Hepatitis C positive. Clinical progression was more rapid in co-infected patients. Moreover, they found the prognostic value of Hepatitis C infection to be significant for both clinical and immunological progression of HIV at early stages of HIV infection. Finally, the ongoing Swiss HIV Cohort Study by Greub et al [47] assessed clinical progression of HIV, and the virological and immunological responses to potent antiretroviral therapy in HIV positive patients with or without HCV infection. They found the probability of progression to a new AIDS-defining event or death to be independently associated with HCV seropositivity and active IDU. However, virological response to antiretroviral therapy and the probability of treatment change were not significantly associated with HCV serostatus.

There are a limited number of studies that have assessed HIV disease progression in patients co-infected with HCV. The discrepancies in these studies are with respect to whether HCV has a deleterious impact on progression to AIDS. There are a number of reasons for the discrepancies in the literature. It is likely that access to highly active antiretroviral drugs in recent years (since late 1996), and the resulting increase in the survival of HIV positive patients constitutes the main reason why there have been discrepancies in results between the initial and more recent studies. Other reasons for the discrepancies may be: 1) differences in study design, 2) differences in the type of patients enrolled and included in the study (haemophiliacs, IDUs, homosexuals), since it has been discovered that there are distinct disease trajectories among different risk groups infected with HIV, 3) small sample sizes, and 5) failure to correct for important confounding variables such as active IDU.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Objectives and Hypotheses

The objectives of this study are threefold:

1) To establish the prevalence of viral Hepatitis C in HIV-infected patients in a Northern Alberta HIV database and to describe the attributes of the patients included in the database.

2) To assess the determinants, risk factors, and impact of HIV disease progression in patients co-infected with HIV and Hepatitis C.

3) To assess the interactions between Hepatitis B Virus, HIV, and Hepatitis C Virus.

Co-infection with Hepatitis C Virus and HIV occurs commonly, and chronic viral Hepatitis C may adversely affect the outcome of patients with HIV infection. Specifically, attention will be on survival in people co-infected with HIV and HCV and whether there is a more rapid or severe disease progression of HIV in co-infected individuals. In the literature, the progression of HIV to AIDS generally was not significantly different in patients co-infected with HIV and Hepatitis C than in patients only infected with HIV. The consensus thus far is that co-infection with HCV does not influence clinical or immunological progression of HIV. However, the lack of association in any study may be the result of a small sample size, and/or the difficulty of differentiating an effect of Hepatitis C from other determinants of HIV disease progression and from the substantial normal variation in the course of HIV disease.

It is the hypothesis of this study that, if an association were to be found, predictive factors for disease exposure would be high-risk demographic factors, such as:

a) aboriginal ethnic status
b) injection drug use (with or without other risk factors)
c) age group (specifically 26-40 years old)
d) low socioeconomic status

Predictive factors for HIV disease progression to any of the four selected HIV endpoints might include:

a) immunologic factors: baseline CD4 cell count <500 and/or baseline CD4 percent <29%
b) ineffective highly active antiretroviral treatment (HAART) for HIV

The first objective of this study is to determine the point prevalence of HCV in an HIV-infected population. Point prevalence is the proportion of individuals in a population who have a specific condition at a point in time. Prevalence is a useful measure for chronic infections such as HIV, HCV, and HBV because it reflects the risk that an individual will have a specific condition.

Once the prevalence and risk factors, if any, for an accelerated HIV disease progression in co-infected patients have been established, they will provide a baseline prevalence measure for analyzing future trends in disease frequency among the population of individuals co-infected with HIV and HCV. Moreover, the findings should provide physicians and administrators with the foundation for inaugurating programs that would be targeted at preventing the transmission of HIV and HCV. The findings might also provide a stronger case for treating Hepatitis C in HIV positive patients.

3.2 Study Design

Because this study makes use of an existing database of patients and there was no data collection involved as a part of the study, it can be considered a retrospective cohort study. In a retrospective cohort study, the investigation is initiated at a point in time after both the exposure and disease (or outcome of interest) have already occurred. Thus, the conduct of this study involves evaluating existing information.

In many respects, this study cannot be viewed as an inception cohort. In an inception cohort, patients should have been identified at an early and uniform point (inception) in the course of their disease (such as when they first developed unambiguous symptoms), so that those who succumbed or completely recovered are included with those whose disease persisted [9]. In this study, a number of patients had not been followed since the inception of their HIV disease. Rather, they had been HIV positive for some time prior to being diagnosed as HIV positive. Thus, by the time these patients were diagnosed as HIV positive, they may have been much further along in the course of their illness than somebody who was diagnosed as HIV positive shortly after they seroconverted for HIV. The apparent risk of progressing to any of the HIV endpoints can be elevated in patients who have not been followed since the inception of their disease. While the failure to start a study of clinical course and prognosis with an inception cohort can have an unpredictable effect on its results, in most cases, the effect would be to make prognosis appear gloomier than it really is [9].

3.3 Ethics

The University of Alberta Research Ethics Board approved the establishment of the HIV database in the early 1980s. The ethics application for this thesis topic has been approved. Ethics approval was based on the following: study procedures are limited to examination of patient, medical, or institutional records, and secondary analysis of data. The research is an epidemiological study, which was carried out at the University of Alberta Hospital site.

The primary ethical issue surrounding this study is one of confidentiality of the patients involved. The database is kept under lock and key and requires password access. In order to maintain confidentiality, the form of the database that was used for this study was stripped of all personal identifiers, specifically the names of the individuals who are entered in the database, prior to being made available to the researchers.

3.4 Description of the Database

The database contains information on all patients who have ever been seen for HIV infection at the University of Alberta Hospital (UAH) and the Sexually Transmitted Disease (STD) Clinic sites, regardless of whether they were initially diagnosed in Alberta or elsewhere. Therefore, it includes patients who were diagnosed as HIV positive outside of Northern Alberta, but later moved to Northern Alberta and were seen for their HIV infection. The database is believed to contain the majority of HIV infected patients in Northern Alberta [65]. It is impossible to know the exact proportion of HIV positive patients the database has captured. However, historically, HIV patient care has been provided largely by members of the Division of Infectious Diseases in Edmonton. There are no physicians or groups providing care to significant numbers of HIV-infected patients who do not share information and, in most cases, care of the patients with the Division of Infectious Diseases. The Provincial Lab generally asks about the involvement of Division members in a patient's care when CD4 cell counts or viral load measurements are requested, and the only two pharmacies that stock HIV antiretroviral drugs apply a similar policy. Therefore, most patients with a diagnosis of HIV infection and virtually all that are receiving therapy are included in the HIV database. The number of individuals with HIV infection who have not been diagnosed is, of course, unknown. However, a recent seroprevalence study in two Edmonton Emergency departments, which both serve high risk populations, found that 80% of HIV-infected individuals, but less than 40% of Hepatitis C-infected individuals had evidence of a prior diagnosis [59]. Thus, <20% of HIV was not previously diagnosed, suggesting that the number of undiagnosed individuals for HIV in Northern Alberta is relatively small. On the other hand, >60% of Hepatitis C was not previously diagnosed, indicating a larger number of undiagnosed individuals for HCV in Northern Alberta.

The STD Clinic site, previously operated by Alberta Health (AH) and now by the Capital Health Authority (CHA), is located in downtown Edmonton, Alberta. This database includes the retrospective entry of cases to as early as 1979. Therefore, the inclusion criterion for this study is HIV infection and entry into the UAH HIV database. This database is maintained by the HIV program at the University of Alberta Hospital site and has been funded by the HIV program with support from LCDC and the Capital Health Authority since 1991. There are no exclusion criteria. HIV disease does not discriminate and this database can be considered representative of the typical HIV seropositive patient.

The database contains various demographic data. See Table 3.2 for a list of all variables included in the database. Risk behaviour for HIV infection is broken down into eight categories: bisexual, homosexual, injection drug user (IDU), heterosexual, transfusion recipient, needle stick exposure, vertical transmission from mother to child, and unknown. There are various combinations of the above risk behaviours. For example, an injection drug user may be bisexual, homosexual, or heterosexual. More than one risk factor may be assigned to an individual, but the risks are ranked according to which, in the opinion of the attending physician, was the most likely route of HIV transmission. Gender is categorized as male or female. There are eight categories for ethnic origin: Aboriginal (includes both Treaty Indians and Metis), Black, Caucasian, Hispanic, Indian, Oriental, unknown, and blank if not recorded. Other demographic data are as listed in Table 3.2. The date first seen refers to the date when a patient was first seen for HIV infection at either the UAH or one of the STD Clinic sites. Age categories were created based on age when first seen (0-14; 15-25; 26-40; ≥41). Height (in cm) and weight (in kg) are flow measurements that have been taken over time. Imprisonment refers to whether or not the patient has ever been incarcerated.

Date of hepatitis B vaccination is indicated, as well as information on Hepatitis B surface antibody and Hepatitis B surface antigen (positive, negative, or not tested). Another variable called Hepatitis B surface antigen/antibody was created. This indicates whether a patient is a) negative for both of Hepatitis B surface antigen or antibody, b) positive for either of Hepatitis B antigen or antibody, c) not tested for either of Hepatitis B antigen or antibody. Carriers are antigen positive, patients who are either naturally immune or have been vaccinated are antibody positive, and patients who have never been infected show neither antigen nor antibody in their serum. Hepatitis C antibody status, CMV status, and toxoplasmosis status are included as either positive, negative, or not tested. Finally, Mantoux test date, pneumovaccination, influenza vaccination, syphilis testing, and physician (both primary care and infectious disease physician) information is available.

There are a number of clinical and laboratory parameters that are tracked over time. These include ALT (alanine transaminase) levels, CD4 cell counts, CD4 percent, HIV RNA levels (after late 1996), and whether or not the patient has ever had an AIDS-defining illness (according to the conditions included in the 1993 AIDS surveillance case definition in Appendix I). Finally, a record of prescription drugs (including highly active anti-retroviral drugs), ongoing conditions, hospital days, new events, and related comments are available.

The information extracted from the follow-up variable CD4 cell count is as follows: minimum CD4 cell count, baseline CD4 cell count, CD4 cell count categories based on baseline CD4 count (0-100; 101-200; 201-500; ≥501), and the HIV endpoint of whether or not the patient ever progressed to a CD4 cell count ≤100. With respect to the CD4 percent, the identical information was extracted; that is, minimum CD4 cell percent, baseline CD4 cell percent, CD4 cell percent categories based on baseline CD4 percent (≤8%; 9-13%; 14-28%; ≥29%), and the HIV endpoint of whether or not the patient has ever progressed to a CD4 cell percent ≤8%. The CD4 cell count and CD4 percent categories correlate with one another and were chosen based on the available literature [5]; the only approximations were the correlation of a CD4 cell count ≤100 and a CD4 percent ≤8%. According to the CDC, a CD4 percent ≥29% corresponds to a CD4 count ≥501; and, a CD4 percent of between 14-28% corresponds to a CD4 count of 201-500. However, there is no approximation given for a CD4 count of 100 and its equivalent CD4 percent value. Thus, a CD4 count of ≤100 and a CD4 percent ≤8% are expected to be close approximates of one another.

The flow information on HIV RNA viral loads (after late 1996), which are measured over time, allowed us to create a variable that was the minimum viral load. Finally, a variable corresponding to effective treatment and date of treatment were created from the viral load information. Effective treatment (yes, no, or missing) was considered to be treatment, which resulted in two consecutive viral loads ≤500 viral particles/mm^3. The date of the first undetectable viral load (≤500) was indicated for these patients. Alternatively, date of onset of HIV therapy was used for patients that have a viral load >500 (i.e. patients who do not have effective treatment). All data in the database are linked by patient identification.

3.5 Variables to Consider for the Study

In analyzing the co-infection of HIV and HCV, one of the objectives of this study is to focus on HIV disease progression in patients co-infected with Hepatitis C Virus. Four HIV endpoints were selected from the administrative HIV database. In addition, there are several general descriptive variables that will be analyzed.

3.5.1 HIV Endpoints

1. AIDS-defining illnesses, such as Pneumocystis carinii Pneumonia (PCP), Kaposi's Sarcoma (KS), and liver disease (resulting from co-infection with HCV). See Appendix I for a complete list of all AIDS-defining conditions that are currently included in the AIDS surveillance case definition [5].
2. Surrogate endpoint: CD4 cell count
3. Surrogate endpoint: CD4%

   Both of these surrogate endpoints are immunological parameters.
4. Death related to HIV/AIDS.

3.5.2 HCV Endpoints

1. Liver disease endpoints (cirrhosis, clinical liver failure, hepatocellular carcinoma).
2. Death from cirrhosis/liver failure.
3. Biopsy (histology).
4. Alanine transaminase (ALT) levels.

3.5.3 Other Variables

1. Gender
2. Risk behaviour.

3. Ethnic status.
4. Socioeconomic status.
5. Alcohol consumption.
6. Age at seroconversion for both HIV and HCV.
7. Drugs (highly active anti-retroviral therapies) and treatment/therapy.
8. Hepatitis B surface antigen/antibody status.
9. Co-morbid conditions and their related drug therapies.
10. Nutritional status or BMI (Body Mass Index).
11. HCV genotype.
12. Virological parameters: a) HIV RNA viral load.
    b) HCV RNA viral load.

The clinical AIDS-defining illnesses that are being used as HIV endpoints have been taken from the 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among adolescents and adults. See Appendix I for a list of conditions included in the 1993 AIDS surveillance case definition. Because prophylaxis and the highly active anti-retroviral drugs (HAART) improve outcomes of HIV-infected individuals, onset of AIDS is considered to be an increasingly poor endpoint. For this reason, viral load will be considered as a measure of treatment effect. Nonetheless, it is important to consider AIDS as an endpoint because HAART has been widely available in Northern Alberta only since late 1996.

Both CD4 percent and rate of change of CD4 percent in an individual have been shown to have significant prognostic value in determining AIDS-free survival time [4]. The CD4 cell count variable is included in the database; the CD4 percent has been recorded in the database for the past 5 years. The CD4 percent will be an important surrogate marker for HIV disease progression in co-infected people. The baseline CD4 percent has proven to have greater prognostic value than the baseline CD4 cell count [4]. For this reason, the baseline CD4 percent should be used as an entry criterion for the study, and the rate of CD4 percent change can be viewed as an indicator of clinical progress and correlated to specific HIV endpoints. The study by Burcham et al. did not find baseline CD8 cell counts and baseline CD8 percent to be independently significant as predictors for the development of AIDS. This is consistent with findings from other studies. Therefore, for the purposes of this study, CD4 count and CD4 percent were used as HIV endpoints. However, measures of the rate of change of CD4 percent were not included in the HIV database. The rate of change of CD4 percent is an indicator of clinical progress that should be considered in future studies.

As mentioned in the objectives/hypothesis section, one of the objectives of this study is to evaluate the HIV endpoints and HIV survival in co-infected patients. It is not feasible, for the purposes of this study, to consider the HCV endpoints and Hepatitis C survival in co-infected patients. This decision was based primarily on the fact that there are very few Hepatitis C endpoints in this study (less than five for each endpoint). This effectively prevents any meaningful statistical analyses from being performed. There are very few endpoints because Hepatitis C has such a long latency of infection before cirrhosis, clinical liver failure, hepatocellular carcinoma, or death endpoints manifest (up to 20 years in some cases).

Biopsy (or histology) is not feasible because it is an invasive procedure that many physicians would recommend only when the information would be used to alter treatment. It is rarely, if ever, available. Finally, ALT levels were measured. However, as mentioned in the literature review, ALT levels do not have a strong correlation with HCV viraemia [15]. ALT levels are mediocre and inconsistent indicators of active disease and inflammation. Furthermore, they are very poor indicators of cirrhosis and liver damage. Therefore, the HCV endpoints have been listed simply as a means to provoke consideration of possible variables and endpoints that could be investigated in future studies.

Age at seroconversion for both HIV and HCV was not always available in the database. Onset of IDU may be an appropriate surrogate for age at seroconversion for HCV because injection drug use and Hepatitis C are so closely related, and within 6 to 12 months of onset of IDU, 80%-90% of IDUs become infected with HCV {Stan Houston, personal communication]. The date first seen was used to measure age at seroconversion for HIV in patients for whom the date first seen is the same as their date HIV seropositive. The variable 'date first seen' in the database is, in fact in most cases, the date first known to the UAH or one of the STD Clinic sites to be HIV antibody seropositive, and not just the date first seen. However, there are patients who present to the UAH or STD Clinic site for the first time and have been previously diagnosed as HIV seropositive elsewhere (i.e. another province). These patients' date first seen is not the same as their date HIV seropositive. Age at seroconversion for these patients is thus calculated using their date HIV seropositive. On the other hand, there are patients who present to the UAH or Clinic site for the first time and are subsequently diagnosed as HIV positive at the time of visit or very shortly thereafter upon receiving a positive diagnostic test result for HIV. These patients' date first seen is therefore the same as their date HIV seropositive.

In the literature, date seropositive and age at seroconversion are usually measured using the midpoint between date seropositive and date last negative. However, these studies tend to be well controlled, and both measurements are thus available. In this database, there are several patients for whom the "date seropositive for HIV" is inaccurate and "date last negative" is unavailable, since for many patients, their positive test was their first test. This can be viewed as another limitation of the database. However, for patients who have an accurate, known date of HIV seropositivity, this date is used to measure age at seroconversion for HIV. For patients who have a previous negative test date, the date seropositive and age at seroconversion are measured using the midpoint between their "seropositive date" and their "date last negative".

Age is a critical factor in any study assessing the disease progression of HIV infected individuals because age plays a very important role in determining outcome [7]. There is a well-documented pattern of smoothly decreasing survival with increasing age at HIV infection. Age is also statistically significantly related to histological grade and stage of HCV [28]. In the study by Delladetsima et al., "cirrhosis" and "moderate chronic Hepatitis" were diagnosed statistically more frequently among patients older than 40 years, while "no" or "mild fibrosis" and "minimal chronic Hepatitis" were more common among patients 40 years old or younger.

The reason for including alcohol consumption is because both excessive drinking and HIV infection have been shown to be independently associated with the finding of cirrhosis in HCV-infected IDUs. To rule out the possible confounding effect of alcohol consumption on HCV disease progression, this variable must be included in the survival analysis. Specifically, we would like to determine if it is alcohol consumption or HIV and HCV co-infection that promotes the liver disease endpoint. Bonacini et al. [60] found 22% of co-infected patients as having histologically-verified alcoholic Hepatitis. However, information on alcohol consumption is not reliably or consistently recorded in the database, and for this reason it is difficult to meaningfully analyze any confounding effects this variable may have.

It is imperative that anti-retroviral drugs be considered in the analysis. Firstly, the drug regimen for treating HIV has drastically changed over the course of the past 15 years. The prognosis for HIV-antibody positive patients undergoing therapy has significantly improved. It has only been approximately 4 years since a highly effective suppressive treatment has been available for HIV and the knowledge to date regarding the long-term benefit of this therapy is not yet known. Prior to this, treatment likely had a small effect. Furthermore, some patients elected not to undergo therapy because of the potential side effects or because of difficulty adhering to the strict, near-perfect regimen required. In addition, in the late 1980s and early 1990s, there was no standard treatment for HCV. The majority of patients were not treated for their HCV. In the HIV database, there were only 16 patients treated for their HCV; they were treated with recombinant Interferon Alpha (INF-α), an antiviral treatment also used for HBV and certain HIV-related complications, namely Kaposi's Sarcoma. None of the patients in the database were documented as receiving ribavirin for their HCV.

HCV genotype is another variable that should be addressed as a useful indicator of HCV treatment response. Genotype varies mainly geographically, with genotype 1 being predominant in Canada. HCV genotype 1 has been associated with elevated HCV viraemia [61]. The increase in HCV viraemia in HIV-antibody positive patients that has been observed and described could perhaps be attributed to the predominance of HCV genotype 1 in these patients. If this is the case, HCV genotype may be a potential confounding variable. The study by Cribier et al. found the increase in HCV viraemia observed in HIV-antibody positive patients was not the result of the distribution of HCV genotype. HIV infection was responsible for an increase in HCV viraemia, irrespective of HCV genotype [42]. HCV genotype 1 has also been associated with more severe hepatic changes and a poor response to interferon therapy [27]. Although genotypes 1a and 1b seem to be less responsive to interferon therapy, no clear association with disease outcome or severity has been demonstrated despite initial suggestions that infection with genotype 1b is more likely to lead to cirrhosis or hepatocellular carcinoma [27]. In light of the above information, HCV genotyping is a fairly recent phenomenon that is quite costly. Furthermore, it is currently unavailable in our Provincial Laboratory and therefore, this variable was not considered.

HIV viral load is an objective indication of whether HIV treatment is effective. For the purposes of this study, we have taken two consecutive undetectable viral loads (≤500) as an indication that HIV treatment has been effective. This variable is extremely important, as it will help establish whether or not there is a masking effect of treatment on HIV disease progression in co-infected patients. Viral load testing has been available, however, only since late 1996. On the other hand, Hepatitis C viral load is currently unavailable at our Provincial Laboratory, but should be considered in future studies.

Finally, primary care physician, ongoing conditions, and drug therapies are available from the database and will be used in any analyses where appropriate.

3.6 Testing for HIV

Testing for HIV-antibody in Northern Alberta is done only in the Northern Alberta Provincial Laboratory, which is located at the University of Alberta Hospital. However, similar techniques apply in the Southern Alberta Provincial Laboratories and in other laboratories in Canada such as in British Columbia, where some patients may have been diagnosed. The details of testing, such as the specific "kit" have evolved somewhat, but the basic process has remained relatively unchanged. An Enzyme-linked Immuno Assay (EIA) screening is performed first; if it is positive, another EIA is carried out on the specimen. Specimens that are positive upon this second testing then undergo Western Blotting, whereby positives are determined by standard criteria. Most patients have had repeat testing performed on a second specimen. Since late 1996, most patients also will have had direct detection and quantification of HIV through viral load testing.

3.7 Testing for HCV

In Northern Alberta, Hepatitis C testing is also performed only at the Provincial Laboratory. The EIA testing has improved significantly since the initiation of testing in December 1989. Currently, a positive EIA is confirmed with a recombinant Western Blot. A serological assay, which tests for antibodies to HCV, is the initial screening test for patients with suspected HCV infection. Currently, there are two types of HCV antibody tests, the EIA and the Recombinant Immunoblot Assay (RIBA). Both of these tests detect antibodies to four HCV antigens from core and nonstructural genes. The first test used to detect anti-HCV, introduced and licensed in December 1989, was a first-generation EIA. This test was not very sensitive, and thus a large number of false-negatives were found. The second-generation EIA, which was introduced in 1992, is a much more sensitive test with a sensitivity of 95% or more [62]. This increased sensitivity combined with the elucidation of identifiable risk groups for Hepatitis C significantly reduced the number of false-negative results. Sensitivity is a measure of the true positive rate, thus by increasing the sensitivity you reduce the number of false negatives. The second-generation EIA tests are reproducible and are suitable for screening low- and high-prevalence populations. It is important to note that the current anti-HCV tests do not distinguish between acute, chronic, or resolved infection.

If the EIA test comes back positive, a confirmatory supplemental Recombinant Immunoblot assay RIBA is performed. This method of administering screening tests in series further reduces the number of false-negative results (i.e. increases sensitivity).

False-negative results occur most frequently in low-prevalence populations, such as the healthy blood donor population. Moreover, the RIBA has a higher specificity than the EIA. In general, serial testing results in an increase in sensitivity as compared to a single test, because a series of positive results is more likely to correspond to true disease. However, specificity may decrease because false-positive diagnoses are more likely.

In other geographical locations, direct detection of HCV RNA by molecular assays is performed as a means to quantify the level of viraemia in an individual patient. There are two types of HCV RNA tests: the reverse transcriptase-polymerase chain reaction (RT-PCR), which can be either quantitative or qualitative, and the branched-chain DNA signal-amplification assay (bDNA), which is quantitative. Although qualitative HCV RNA testing by RT-PCR is viewed as the most sensitive test, it has not been standardized and is not considered essential to patient management [62]. When used, qualitative measurements of HCV RNA are useful for monitoring patients on antiviral therapy. Qualitative PCR testing has only been in clinical use at the Provincial Lab for 1 year; quantitative testing is not yet available.

Because alanine aminotransferase (ALT) levels are elevated in a majority of patients with HCV, these liver enzymes must be monitored throughout the course of infection. ALT levels are known to fluctuate over time in HCV patients, and thus a single determination of ALT is not a reliable diagnostic test, nor is it an accurate indication of histological activity or disease severity. The only reliable means of confirming the diagnosis of Hepatitis C and evaluating disease severity is by means of a liver biopsy. A liver biopsy will directly assess the grade and stage of liver disease, as well as eliminate the possibility of other forms of liver disease. Liver biopsies are not routinely performed for patients in this database.

3.8 Sample Size

The number of registrants in the database is n=1,276; all of these patients are HIV-antibody positive. However, only 564 of these patients have been tested for HCV; 326 were found to be HCV positive and 238 are HCV negative. It is important to note that patients first seen before early 1990 could not have been tested for HCV when they were first seen. Therefore, these patients may have been tested later in the course of their care, and some patients in the database died before Hepatitis C testing ever became available.

Patients are generally tested for HCV only if they are considered to be in a high risk group for HCV transmission. It is primarily injection drug users, and transfusion recipients who received pooled blood products before HCV screening was available that fall into this high risk category. This will tend to overestimate the association between IDU and HCV, and result in missing an unknown, but probably small number of HCV infections. Thus, those individuals who are not tested for HCV are unlikely to be HCV positive, provided they gave an accurate account of their risk behaviour status. For obvious reasons, it is sometimes difficult to determine whether a patient has a history of IDU, even if it was only once. Some individuals are ashamed of this behaviour and will not admit to it, but a non-judgemental atmosphere prevails at the HIV clinic so that discussion of IDU-related issues is generally quite open. If these individuals were to be tested for HCV, it is quite possible they may be positive for HCV. If this were the case, the actual number of HCV positive patients would be an underestimate of the true number. However, it is unlikely that many other individuals not tested for HCV would be positive.

3.8.1 Sample Size and Power Calculations

Sample size and power calculations were performed for the proportional-hazards regression model, according to Schoenfeld [63]. Table 3.1 gives the sample sizes required to detect selected hazard ratios with 80% power and a Type 1 error $\alpha=0.05$ (two-sided).

The parameter estimates needed for the sample size calculations were obtained 'post hoc' from the present study, based on the KM analyses with Hepatitis C as the sole predictor variable and CD4 cell count ≤ 100 as the HIV endpoint.

In the present study, when Hepatitis C was entered as the single main effect in the Cox regression for the HIV endpoint of a CD4 count ≤ 100, a HR of 1.4 was obtained. The sample size for this regression model was 161 and there were only 17 events. In order to have 80% power to detect a hazard ratio as small as 1.4, 1,920 patients would have been required. Moreover, the number of events required would have been 538. In the final Cox model chosen for the HIV endpoint CD4 count ≤100 (Model 3, Table 6.4), a HR of 2.2 was obtained for Hepatitis C status. This value, however, did not attain statistical significance. According to Table 3.1, in order to have adequate power to detect a HR of 2.0 and 2.5, respectively, 364 and 183 patients would have been needed for the analyses. This study, however, did have adequate power to detect a HR of 3.0 or larger. It is likely that the large p-values obtained in the Cox analyses can be attributed to the small sample size in this study. It is important to note that, had there been a large enough sample size and power for this study, the results may have been statistically significant.

3.9 Statistical Analyses

In analyzing this database, various descriptive analyses such as crosstabulations and frequency distributions were performed in addition to analyses examining how HIV and Hepatitis C co-infection status correlates with a number of variables over calendar year. Finally, a number of Cox regression survival analyses were performed.

Various line graphs and stacked bar graphs were constructed to describe the database. The whole cohort was used to describe the proportion of patients by gender, by risk behaviour, by age group, by Hepatitis B surface antigen/antibody status, by Hepatitis C antibody status, and by ethnic status. See Figures 4.1 to 4.6 respectively. This part of the descriptive analysis captured information from the entire database. Next, the database was limited to patients with Hepatitis C and the years 1992-1999. The following graphs were plotted: distribution of patients with Hepatitis C by ethnic status, by risk behaviour, by age group, and by gender. These analyses used only the portion of the database from January 1, 1992, based on the date when a patient was first seen, because HCV testing did not become widely available until 1990. Moreover, a time lag must also be accounted for because the test that was available in 1990 was relatively inaccurate and clinicians were not yet aware as to which populations would more likely be associated with Hepatitis C (e.g. IDUs). The data reflected this natural break of 1992, indicating that

HCV testing, in fact, commenced fully in 1992. Finally, the distribution of non-IDUs by Hepatitis C antibody status (whole cohort), the distribution of IDU by Hepatitis C antibody status (1992-1999), and the distribution of Hepatitis B surface antigen positive patients by risk behaviour (whole cohort) were graphed. These analyses were performed using the statistical software package SPSS.

3.10 Selection of the Cohort

Patients have been entered into the Northern Alberta HIV database since as early as 1979. All patients who are HIV positive and have been seen at the University of Alberta Hospital (UAH) or STD Clinic Sites for their HIV infection are entered into the database, regardless of whether they were initially diagnosed in Northern Alberta or elsewhere. Inclusion and exclusion criteria for each of the cohorts considered only differed depending on which analyses were being performed.

3.10.1 Inclusion and Exclusion Criteria for the Descriptive Analyses

All 1,276 patients in the database were used for the descriptive analyses, making it possible to establish the characteristics of the entire cohort. With respect to these descriptive analyses, graphs and their corresponding tables illustrate the pattern of events over calendar time regarding specific variables. There were two types of graphs and tables:

1) Graphs and tables depicting the pattern of events over calendar time for the whole cohort.

2) Graphs and tables depicting the pattern of events over calendar time for HIV and Hepatitis C co-infected patients. Because these graphs and tables evaluate co-infected individuals, it was necessary to include patients only from 1992 onwards, based on the date when the patient was first seen. Firstly, HCV testing was not widely available until December 1989, but more importantly a more sensitive, second generation immunoassay was not available until 1992. There was a significantly large proportion of false-negative tests in the early testing era for HCV. Therefore, to eliminate this

testing bias, patients who tested positive during 1990 and 1991 were excluded in these analyses.

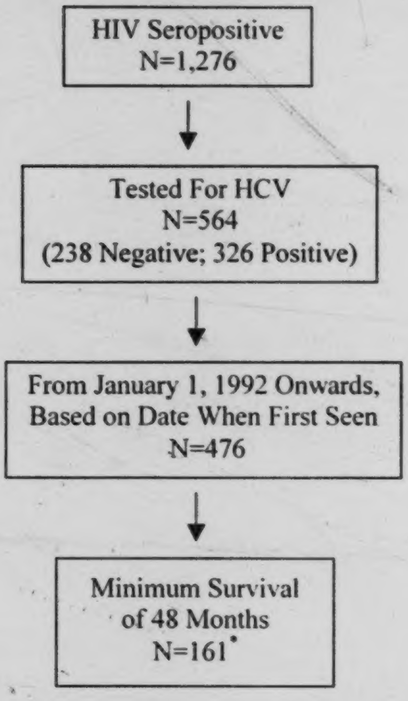
3.10.2 Inclusion and Exclusion Criteria for the Survival Analyses.

Prior to performing the Cox regression, the first step was to obtain Kaplan-Meier survival curves. Survival time for a study depends on the endpoint of the study; in this study, there are four HIV endpoints or outcomes (CD4 cell count, CD4 percent, AIDS-defining illness, and death). Because there are four endpoints, there are four different survival times for each patient, all of which were calculated based on whether a patient was either censored or progressed to the endpoint.

Survival time is calculated as the length of time an individual is under observation; that is, from start time until the endpoint is reached or censoring occurs. Date HIV seropositive was chosen as the start time because there were very few missing values and date seropositive is the closest approximation to when a patient is known to first have HIV for the majority of patients in the database. Date seropositive is also the closest, if not exact, date when a patient first began observation and was entered into the database for the majority of patients. It is important, however, to note at this point that many patients may have been HIV positive for several months or years prior to actually knowing and being diagnosed as HIV seropositive because for whatever reason they had not yet been tested. The end date chosen was December 31, 1999. For patients who progressed to an HIV endpoint, the survival time was calculated by subtracting the date the patient was HIV seropositive from the date corresponding to when the event occurred. For patients who did not progress to an HIV endpoint, the survival time was calculated by subtracting the date they became HIV seropositive from the end date (December 31, 1999). All survival times were calculated in months.

When the survival times had been calculated for all four HIV endpoints, a closer examination revealed that there were a number of negative survival times, as well as some very short survival times. An explanation for these negative or very short survival times is that many patients were not tested for HIV and subsequently diagnosed as HIV seropositive until they had already reached one or more of the endpoints. Negative survival times were a result of patients showing up very late in the course of their illness; that is, these patients had progressed to one of the HIV endpoints prior to being tested positive for HIV. Some patients with HIV lead chaotic lifestyles and they do not seek medical help when it is necessary. Furthermore, because of the infectious nature of HIV, it is often difficult to determine exactly when a person first became exposed and subsequently seroconverted for HIV. This is an issue that one faces with many infectious and chronic diseases; thus, the data in this database can be considered left-censored. Left-censored data result from not knowing when a patient was first exposed to a disease. Moreover, this cohort was not an inception cohort. Patients were not assembled into the cohort at an early and uniform point in the course of their HIV disease. Rather, patients were entered into the cohort (database) upon having a known HIV positive diagnosis. This HIV diagnosis did not always occur at an early and uniform point in the course of the patients' HIV disease.

Because of the negative and very short survival times, patients were selected for the Kaplan-Meier and Cox regression analyses based on the following approach: patients were selected only if they had survival times ≥48 months. While it is possible that a patient who has an accurate date for HIV seroconversion may, in fact, reach one of the chosen HIV endpoints within the first 48 months after HIV seroconversion, it would be quite rare. Thus, upon close examination of the survival times for each patient, it was our conclusion that those patients, who progressed to one of the HIV endpoints within the first 48 months following their recorded HIV seroconversion date, were unlikely to have an accurately recorded date for HIV seroconversion. In other words, these patients were likely to have been HIV seropositive for quite some time prior to their testing positive for HIV, but were not aware of their HIV infection. Thus the overall inclusion criteria for the survival analyses are as follows (see top of page 44):


HIV Seropositive
N=1,276
Tested For HCV
N=564
(238 Negative; 326 Positive)
From January 1, 1992 Onwards, Based on Date When First Seen
N=476
Minimum Survival of 48 Months
N=161[*]

The first set of inclusion criteria required that patients be HIV positive. Patients are only entered into the database if, in fact, they are HIV positive. Thus all 1,276 patients in this database are HIV positive. From this point, patients must have been tested for Hepatitis C. There were 564 HIV positive patients that were tested for Hepatitis C, 238 of whom were Hepatitis C negative and 326 of whom were Hepatitis C positive. The third inclusion criterion states that patients were selected from only the year 1992 onwards, based on the date when the patients were first seen by an infectious disease physician. This cut-off was placed at January 1, 1992 to allow for the lag time in Hepatitis C testing, as previously mentioned. At this point, the sample size was reduced to 476 patients. The final inclusion criteria required that patients be under observation for at least 48 months. This effectively further reduced the sample size to 161 patients (for the analyses using the HIV endpoint CD4 count ≤100).

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[*] N=161 is the sample size for the HIV endpoint CD4 count ≤100. This is the HIV endpoint used for the large majority of Cox regression analyses.

In addition, consistent with the 48-month criterion, survival times for the 161 cohort members were recalculated with the start time taken to be at the 48-month point. Using these newly calculated survival times, KM curves and the Cox analyses with Hepatitis C entered as the single main effect, were performed. The reason for redoing these analyses, using different survival times, was to determine whether the HRs and KM curves would change. The results, in fact, were identical to the original analyses.

Although, the above was the selection criterion for all survival analyses from this point onwards, Kaplan-Meier curves are also shown for time periods of: 1) all survival times, 2) survival times ≥24 months, 3) survival times ≥36 months, and 4) survival times ≥60 months. This allows the reader to visualize why the cut-off of 48 months was chosen, as further described in Chapter 5. By selecting patients only if they had a minimum survival time of 48 months, we lost the ability to analyze those patients who reached an HIV endpoint at a very early stage (i.e., prior to 48 months). Also, by definition, requiring patients to survive 48 months means that there are no patients in the analyses who had a start date after December 31, 1995. By excluding patients who did not survive a minimum of 48 months, we effectively created a bias by selecting healthier patients for the study. The patients who were excluded came to our attention because they were reaching the HIV endpoints much sooner than the average duration of HIV disease progression to whichever endpoint they progressed to (whether it be a CD4 count ≤100, a CD4 percent ≤8%, an AIDS-defining illness, or death). It is anticipated that, by creating a bias and selecting a healthier group of patients, it will effectively decrease the measure of effect (hazard ratio) towards the null (i.e. it will be an underestimate of the true measure of effect). Although there was a bias introduced by selecting patients based on their outcome, it would be unreasonable to include these patients in the analysis. By including them we would be assuming that all of the patients who progressed to an HIV endpoint very early on (i.e., within the first 48 months following HIV seroconversion), had an accurate known date of HIV seroconversion, when in fact this was not the case.

TABLE 3.1: SAMPLE SIZE CALCULATIONS FOR SELECTED HAZARD RATIOS.

Hazard Ratio	Total Sample Size Required	Number of Events
1.4	1,920	538
2.0	364	127
2.5	183	73
3.0	113	50
5.0	42	24

TABLE 3.2: COMPLETE LIST OF ALL VARIABLES IN THE HIV DATABASE

Demographics	Immunologic Parameters	Other Disease Status	HIV Endpoints	Miscellaneous
Patient ID number	Baseline CD4 cell count (and date)	Cytomegalovirus status	AIDS-defining illness (and date)	Minimum HIV viral load (and date)
Birth date	Baseline CD4 percent (and date)	Hepatitis B surface antibody status	CD4 cell count ≤100 (and date)	Minimum AST level (and date)
Death date	Minimum CD4 count (and date)	Hepatitis B surface antigen status	CD4 percent ≤8% (and date)	Treatment date
Age at death	Minimum CD4 percent (and date)	Hepatitis B surface antigen/antibody status	Death	Effective treatment
Date first seen	CD4 cell count categories	Hepatitis B vaccination (and date)		Ongoing conditions
Age when first seen	CD4 percent categories	Hepatitis C antibody status		Primary Care physician
Date seropositive for HIV		Mantoux test date		Infectious disease physician
Date last negative for HIV		Mantoux test result		Hospital days
Height		Toxoplasmosis status		New events
Weight		Syphilis test result		
Gender		Pneumovaccination		
Risk behaviour		Influenza Vaccination		
Ethnic status				
Imprisonment				
Highest grade completed				

CHAPTER FOUR

RESULTS - DESCRIPTIVE

This chapter provides the results of the descriptive analysis of the study cohort as a whole, as well as for various subgroups within the cohort. A number of characteristics of the study population are identified. In addition, prevalence is examined, the effect of HIV treatment, and the association of Hepatitis B Virus with both HIV and HCV. Finally, a series of graphs and tables are provided to demonstrate how HIV and Hepatitis C co-infection correlates with a number of variables over time (by calendar year). These graphs and tables are also provided for the whole cohort.

4.1 Characteristics of the Study Population

Select demographic characteristics are shown in Table 4.1. Demographic characteristics are given for the entire cohort of 1,276 HIV patients, as well as for the 326 patients co-infected with Hepatitis C, and the 950 patients infected only with HIV.

4.1.1 Characteristics of the Whole Cohort

There are 1,276 patients in the whole cohort, all of whom are HIV-antibody positive. For these patients, the median age is 32.8 years, 81.3% are male, and the largest risk behaviour group is that of homosexual and bisexual transmission, which comprised 46.6% of the cohort. IDU accounted for 33.7%. A smaller proportion was accounted for by heterosexual transmission (14.4%), and 5.3% indicate vertical transmission or a blood transfusion as the source of their HIV infection. Caucasians are the largest ethnic group in the cohort with 67.7% and Aboriginals are second with 18.7%. Only 11.0% of the cohort has been incarcerated at some point in their lives. However, it is important to note that the information on imprisonment was very inconsistently recorded and captured, in that there was a major underreporting, especially earlier on the epidemic (i.e. prior to 1990), and thus, this is an underestimate. Finally, 37.2% of patients have had one or more

AIDS-defining illnesses, and 72.9% of the cohort was still alive as of December 31, 1999.

The mean baseline CD4 cell count for the cohort of 1,276 patients is 425.8, with 32.7% of patients falling within the 201-500 cell count category. Furthermore, the mean baseline CD4 percent is 21.6, with 31.0% of patients falling in the 14-28% CD4 percent category. Of those patients for whom HAART treatment information was available (50.0%), a larger proportion (58.6%) have responded to the therapy, which is reflected by their HIV viral load being <500 on two consecutive measurements.

4.1.2 Characteristics of Patients Only Infected with HIV

Within the group of 950 patients infected only with HIV, the median age is 32.9 years, 86.2% are male, and a large percentage (60.9%) indicate bisexual or homosexual transmission. Only 18.4% was accounted for by heterosexual transmission, and a smaller proportion (14.7%) are injection drug users. The group of HIV positive patients is primarily Caucasian (72.6%), and a very small percentage (3.3%) have been in jail. Almost half (44.7%) of HIV mono-infected patients have had an AIDS-defining illness and 66.4% were alive as of December 31, 1999.

The mean baseline CD4 cell count for the 950 HIV positive patients is 403.5, with 28.3% of the individuals falling within each of the 101-200 and 201-500 CD4 cell count categories. Furthermore, the mean baseline CD4 percent is 19.2, with 26.9% of patients falling in the 14-28% CD4 percent category. Of those patients for which treatment information was available (39.5%), a larger proportion (67.0%) have responded to therapy, which is reflected by their HIV viral load being <500 on two consecutive measurements.

4.1.3 Characteristics of Patients Co-Infected with HIV and HCV

Once the cohort was limited only to HIV and HCV positive patients (n=326), the following descriptive results were obtained. The median age is 32.7 and 66.9% of co-infected patients are male. Thus, there is an increase in the proportion of patients that are female (33.1%), as compared to the whole cohort (18.7%) and those infected only with HIV (13.8%). From above, there were 33.7% (430/1276) of the patients that reported a history of injection drug use (IDU) in the whole cohort. Furthermore, the overall prevalence of HCV infection in the cohort was 25.6% (326/1276). It is interesting to note that only 67.4% (290/430) of those patients with a history of IDU in the whole cohort had a positive test for HCV. Perhaps this is because 22.6% (97/430) of patients with a history of IDU have not been tested for Hepatitis C. However, of those IDUs tested for HCV, the proportion positive was 87.1% (290/333). The number of IDUs that are positive for HCV (67.4%) is smaller than expected, considering that up to 90% of injecting drug users are positive for antibodies to HCV [14, 15]. Therefore, if the 97 patients that are IDU who were not tested for HCV had been tested, it is anticipated that a large proportion would be positive for HCV-antibodies. This, in effect, would increase the number of IDU that are positive for HCV.

As expected, virtually all co-infected patients (89.0%) are IDUs; there were 12 people (3.7%) who reported transfusion as the source of infection. There is a large increase in the number of Aboriginals (35.3%) among the co-infected strata, compared to the group of patients infected only with HIV (12.9%). However, Caucasian ethnicity still predominates with 53.4%. Furthermore, a much larger proportion (33.4%) has been incarcerated at some point in time. In fact, the group of co-infected patients has the largest proportion of individuals who have been incarcerated at some point in their lives. Once again, however, recall that this may be an underestimate due to imperfect capture of prison data. A smaller number of people (15.3%) have had an AIDS-defining illness and 91.7% of co-infected people are still alive, as compared to the patients only infected with HIV (44.7% have had an AIDS-defining illness, and 66.4% are still alive).

The co-infected group has the smallest proportion of patients diagnosed with an AIDS-defining illness and the largest proportion of patients that are still alive, reflecting more recent infection with HIV in this group. Because the co-infected group has, on average, a more recent HIV infection than the group mono-infected with HIV, the stage of their HIV will be substantially different. The smaller number of people with an AIDS-defining illness and the fact that most co-infected patients are still alive is probably the result of a few factors. The main reason is that patients who were infected with HIV a long time ago were mostly homosexual and Hepatitis C negative, whereas recent HIV positive patients are more often IDUs and Hepatitis C positive. Co-infection of HIV with Hepatitis C is a fairly recent phenomenon, because of the recent change in the local HIV epidemic to IDUs. Therefore, the progression of either HIV or HCV disease will not have, in most cases, taken its full course. In addition, the emergence of HIV and HCV co-infection has coincided with significant improvements in HIV therapy (HAART). It is anticipated that these highly active antiretroviral drugs will improve the quality and length of many people's lives.

The mean baseline CD4 cell count for the 326 co-infected patients is 483.3 (as compared to a mean baseline CD4 cell count of 403.5 in the group only infected with HIV), with the largest proportion (45.4%) of the individuals falling within the 201-500 CD4 cell count category. Furthermore, the mean baseline CD4 percent is 26.4%, with 42.6% of patients falling in the 14-28% CD4 percent category. Of those patients for which treatment information was available (80.6%), that is, those patients who received HIV therapy a larger proportion (52.9%) have not responded to therapy, which is reflected by their HIV viral load not having reached a value ≤500. In fact, 19.3% of the co-infected group has not received any HAART therapy. Although patients who are co-infected present at baseline with higher CD4 cell counts and percentages, a very large proportion have not responded well to therapy as indicated by their HIV viral load counts being >500. This is likely a reflection of the fact that co-infected patients are mainly IDUs and, as such, IDUs predictably have more problems with adherence to complex drug regimens.

4.2 The Effect of HIV Treatment

To assess the effect of HIV treatment (highly active antiretroviral therapies) in co-infected patients versus those who are infected only with HIV, a series of 2X2 tables were constructed for each HIV endpoint. These crosstabulations provided a crude assessment relating each of the HIV endpoints to whether or not the patients had effective treatment. Crosstabulations were performed for three groups of patients: 1) the HIV and Hepatitis C co-infected patients (n=326), 2) the HIV positive patients who have been tested for Hepatitis C but are negative (n=238), and 3) the HIV positive patients who have not been tested for Hepatitis C (n=182). From the list of possible HIV endpoints previously described, four HIV endpoints were selected for the purpose of this study: 1) having one or more of the AIDS-defining illnesses as listed in Appendix I; 2) death; 3) a CD4 cell count ≤100; or, 4) a CD4 percent ≤8%. These 2X2 tables were all stratified by HCV status, and each assessed one of the above-stated HIV endpoints with HIV treatment (yes or no).

The main reason for assessing the impact of treatment is because treatment of HIV will have an effect on the outcome of HIV only if the respective treatment works. Viral load was used as a reliable and biologically sound indicator of treatment. This is not a perfect measure, but it is an objective, numerical estimate. The premise behind using viral load is that the longer one goes with an undetectable viral load, the more effective the treatment is. The definition of effective treatment used for the purposes of this study is that the patient has two consecutive undetectable viral loads. Therefore, their viral load measurement must be ≤500 on two consecutive tests. Viral load testing is expensive and thus is normally done approximately every 3-4 months in patients receiving treatment.

4.2.1 HIV Treatment in the Co-Infected Group.

The first series of crosstabulations performed comprise the strata of individuals co-infected with HCV. These crosstabulations are provided in Table 4.2. In the group of patients that did not have effective treatment (viral load >500), 9.6% (24/250) died, 17.6% (44/250) have an AIDS-defining illness as described in Appendix I, 14.4% (36/250) have a CD4 percent ≤8%, and 16.4% (41/250) have a CD4 cell count ≤100. These numbers can be compared to the group of patients that did have effective treatment (viral load ≤500): 3.9% (3/76) died, 7.9% (6/76) have an AIDS-defining illness as described in Appendix I, 9.2% (7/76) have a CD4 percent ≤8%, and 7.9% (6/76) have a CD4 cell count ≤100.

The effect of HIV treatment in co-infected patients was tested using the chi-square test. See Table 4.2.1 for the chi-square test results. Within the strata of individuals co-infected with HIV and Hepatitis C, the only statistically significant difference found between effective treatment and any of the four HIV endpoints, was with the HIV endpoint AIDS-defining illness. AIDS-defining illness barely achieved significance, with a p-value of 0.05. Thus, based on the p-value, the HIV endpoint AIDS-defining illness and HIV treatment status can be considered associated with one another. One would expect that patients who do not have effective HIV treatment are more likely to progress to the HIV endpoint AIDS-defining illness.

4.2.2 HIV Treatment in the HIV Mono-Infected Group.

The second series of crosstabulations performed comprise the strata of individuals infected only with HIV (HCV negative, n=238). These results are displayed in Table 4.3. In the group of patients that did not have effective treatment (viral load >500), 23.9% (33/138) died, 36.2% (50/138) have an AIDS-defining illness as described in Appendix I, 26.8% (37/138) have a CD4 percent ≤8%, and 35.5% (49/138) have a CD4 cell count ≤100. These numbers can be compared to the group of patients that did have effective treatment (viral load ≤500): 4.0% (4/100) died, 32.0% (32/100) have an AIDS-defining illness as described in Appendix I, 30.0% (30/100) have a CD4 percent ≤8%, and 32.0% (32/100) have a CD4 cell count ≤100.

The effect of HIV treatment in patients infected only with HIV was also tested using the chi-square test. See Table 4.2.1 for the chi-square test results. Within the strata of individuals infected only with HIV, there was a highly statistically significant difference found between effective treatment and the HIV endpoint death (p-value = 0.000). Therefore, the HIV endpoint death and HIV treatment status can be considered associated with one another; it is likely that patients who do not have effective HIV treatment are more inclined to progress to the HIV endpoint death.

4.2.3 HIV Treatment in the HIV Mono-Infected Group That Has Not Been Tested For Hepatitis C.

The last series of crosstabulations performed comprise the strata of individuals mono-infected with HIV, but who have not been tested for HCV (n=182). These crosstabulations are provided in Table 4.4. In the group of patients that did not have effective treatment (viral load >500), 21.3% (13/61) died, 27.9% (17/61) have an AIDS-defining illness as described in Appendix I, 33.3% (19/57) have a CD4 percent ≤8%, and 31.6% (18/57) have a CD4 cell count ≤100. These numbers can be compared to the group of patients that did have effective treatment (viral load ≤500): 3.3% (4/121) died, 28.9% (35/121) have an AIDS-defining illness as described in Appendix I, 39.5% (47/119) have a CD4 percent ≤8%, and 35.0% (42/120) have a CD4 cell count ≤100.

In the group of patients who are HIV-antibody positive but have not been tested for Hepatitis C, some interesting results appeared; in fact, there was a substantial number of patients who reached the selected HIV endpoints even though they had effective HIV treatment. For those patients who responded to HIV treatment (i.e., viral load ≤500), 3.3% (4/121) died, 28.9% (35/121) had an AIDS-defining illness, 39.5% (47/119) had a CD4 percent ≤8%, and 35.0% (42/120) had a CD4 cell count ≤100. These results are similar to those found in the group of patients who are HIV positive and negative for Hepatitis C. Thus, we might conclude that the majority of these patients, had they been tested for HCV, are likely negative.

The effect of HIV treatment in patients infected with only HIV who have not been tested for Hepatitis C, was also tested using the chi-square test. See Table 4.2.1 for the chi-square test results. Within the strata of individuals infected only with HIV and not tested for Hepatitis C, there was a highly statistically significant difference found between effective treatment and all of the HIV endpoints. All p-values were < 0.001. Therefore, each of the HIV endpoints and HIV treatment status can be considered associated with one another. The interpretation is that patients who do not have effective HIV therapy are more likely to progress to each of the four HIV endpoints than are patients who have effective HIV therapy.

Based on the above crosstabulations, it appears that having HCV is protective. For example, we can compare the proportion of patients that had an AIDS-defining illness and responded to treatment (i.e., viral load ≤500) in the co-infected group as compared to those who are infected only with HIV. In the group that is only HIV positive, 32.0% (32/100) had an AIDS-defining illness, whereas only 7.9% (6/76) had an AIDS-defining illness in the co-infected group. Similar comparisons can be made with two other endpoints: the CD4 percent, and the CD4 cell count, where in both cases the co-infected group appears to be better off.

These findings are presumably related more to timing and duration of HIV infection, since therapy has been controlled. A large proportion of the group of patients that are HIV mono-infected supposedly became infected earlier on in the epidemic and prior to the advent of the HAART therapy, and thus they have had HIV for a longer time. This may account for the larger percentage of endpoints achieved compared to those patients that are co-infected. This points to the need to consider length of time under observation (i.e., survival analysis), as well as the role of calendar time effects. Both of these are explored in detail throughout the remainder of this study.

4.3 The Interactions Between Hepatitis C Virus, Hepatitis B Virus, and HIV

The interaction between HCV and HBV is of considerable interest. Both the number of patients that are Hepatitis C positive and have Hepatitis B surface antigen or antibody, and the number of patients that are Hepatitis B surface antigen or antibody positive and have Hepatitis C were determined. These interactions can be seen in Table 4.5. A total of 162 patients were Hepatitis B surface antigen or antibody positive out of a total 326 Hepatitis C positive patients (162/326); therefore, 49.7% of Hepatitis C positive patients have Hepatitis B surface antigen or antibody. A very large proportion (88.3%) of these Hepatitis C positive patients that are Hepatitis B surface antigen or antibody positive have a history of IDU. On the other hand, 162 patients were HCV-positive out of a total 501 Hepatitis B surface antigen or antibody positive patients (162/501); therefore, 32.3% of Hepatitis B surface antigen or antibody positive patients are HCV-positive.

A small percentage of patients in the whole cohort have been vaccinated against Hepatitis B (4.2%). It is important to note that information on Hepatitis B vaccination is poorly documented and recorded in the database. Moreover, virtually always, Hepatitis B serology is taken at entry to the clinic so that even those vaccinated against HBV would usually have been vaccinated after receiving those results; the implication being that Hepatitis B positive antibodies will in a large majority cases reflect exposure rather than vaccination. This can be compared to the 39.3% (501/1,276) that are Hepatitis B surface antigen or antibody positive in the whole cohort.

Only 3.7% of patients that are HIV positive (n=950) have been vaccinated against Hepatitis B, and 35.7% (339/950) are positive for either Hepatitis B surface antigen or antibody. There are 238 patients that are HIV positive and HCV-antibody negative. Within this group, 3.8% have been vaccinated against Hepatitis B, and 35.3% (84/238) are positive for Hepatitis B surface antibody.

In the HIV and HCV co-infected group, only 5.5% have been vaccinated against Hepatitis B. It is interesting, although not surprising, that the HIV and HCV co-infected group showed the largest proportions of Hepatitis B surface antigen and antibody positive patients (49.7% or 162/326). This suggests that these patients became positive to Hepatitis B via IDU behaviour. As previously indicated, it is likely that many patients co-infected with HIV and HCV lead very chaotic lifestyles and may be less likely to practice safe sex. This, combined with the fact that a large majority of this group of patients is IDUs, would suggest that they have an increased risk of contracting HBV.

4.4 Analysis of Variables Over Time.

In these analyses, selected variables are examined in two groups of patients over time (by calendar year). These groups of patients are 1) the whole cohort, and 2) those patients co-infected with HIV and HCV.

4.4.1 Whole Cohort

This first collection of graphs and tables makes use of the entire cohort (n=1,276) to ascertain how the distribution of certain variables changed over calendar time.

The gender distribution graph using the whole cohort indicates that the proportion of males in all years is larger than that of females. See Figure 4.1 and Table 4.6. However, beginning in 1985, the proportion of females in relation to males increased, and by 1999, the ratio of males to females was approximately 70:30. This shift in demographic characteristics may be related to the decrease in the number of homosexual and bisexual people becoming infected with HIV and an increase in the number of heterosexuals and IDUs, especially women, becoming infected.

The distribution of risk behaviour using the whole cohort shows that there was a sharp decrease in the proportion of bisexual and homosexual men from 1985-1999, whereas there was a steady increase in the proportion of injection drug users becoming infected with HIV in the database. See Figure 4.2 and Table 4.7. There was also a smaller increase in the proportion of heterosexuals. However, vertical and transfusion-related risk behaviour remained relatively constant and accounted for a very small proportion in all years. The sharp decrease in the proportion of bisexual and homosexual people becoming infected may be attributed to the increased awareness in these risk groups with respect to HIV. Earlier in the HIV epidemic, HIV was often referred to as a "gay disease" because the largest proportion of HIV-infected patients were associated with this risk behaviour group. However, as the epidemic unfolded and public health efforts to control and manage this disease were targeted at this group of people, it became evident that HIV was increasingly being transmitted via other routes, namely IDU and heterosexual sex.

Within the whole cohort, the predominant age groups are 26-40 years old, ≥41 years old, and 15-25 years old. See Figure 4.3 and Table 4.8. The largest proportion is accounted for by the 26-40 year old age group, which comprised approximately 60% (744/1223) of the registrants in the database.

The majority of patients in the database have been tested for both Hepatitis B surface antigen and antibody (1034/1225). See Figure 4.4 and Table 4.9. Testing for HBV became more routine, beginning around 1986. Patients are categorized as not tested, positive for one of Hepatitis B surface antigen or antibody, or negative to both Hepatitis B surface antigen and antibody. Based on the distribution of Hepatitis B antigen and antibody status in the whole cohort, it appears as though there are approximately equal proportions of patients who are negative for both Hepatitis B surface antigen and antibody (549/1225), and positive for either Hepatitis B surface antigen or antibody (485/1225). A smaller proportion of patients have not been tested for either Hepatitis B surface antigen or antibody (15.6%).

Since 1992, there has been an increasing trend in the number of individuals being tested for Hepatitis C. See Figure 4.5 and Table 4.10. In 1992, only 17.9% (19/106) of patients entered into the database were being tested; however, by 1999, 90.0% (86/96) of patients were being tested and 62.8% (54/86) were positive. The number of Hepatitis C positive patients is primarily a reflection of the number of injection drug users in the cohort, as Hepatitis C positivity and injection drug use are closely related. However, it is also a reflection of the increased testing on behalf of physicians.

The cohort is overwhelmingly of Caucasian (69.6%) and Aboriginal (17.8%) ethnicity. See Figure 4.6 and Table 4.11. There was a steady decrease in the proportion of Caucasian registrants and a steady increase in the proportion of Aboriginal registrants from 1985-1999.

4.4.2 HIV and HCV Co-infected Patients

For the following graphs, the cohort was limited to Hepatitis C positive patients and the years 1992-99.

The distribution of patients with Hepatitis C by ethnic status indicates that the majority of co-infected patients are Aboriginal (35.9%) or Caucasian (53.9%). See Figure 4.7 and Table 4.12. There was a decrease in the proportion of Caucasian patients from 1992 until

1996, followed by an increase thereafter. By contrast, Aboriginal patients increased in proportion from 15.4% in 1992 to 52.9% in 1996 and then decreased to 29.6% in 1999. Blacks, Hispanics, Indians, and Orientals accounted for a very small proportion of patients co-infected with HIV and Hepatitis C in all years.

As expected, the distribution of Hepatitis C positive patients by risk behaviour indicates that consistently 80-90% of HCV positive patients are injection drug users. See Figures 4.8 and 4.8a, and Tables 4.13 and 4.13a. It is important to note here there is a detection bias in terms of identifying people as injection drug users. Specifically, risk group classification is not independent of Hepatitis C antibody status. If a physician knows or suspects that a patient is an injection drug user, it is highly likely that the patient will be tested for Hepatitis C, than if there is not a history of IDU behaviour.

The age group distribution in Hepatitis C positive patients is similar to that of the whole cohort. See Figure 4.9 and Table 4.14. The majority of patients fall into the 26-40 year old age category (184/284). The remainder of patients are split quite evenly between the 15-25 and >=41 year old age groups. This finding is somewhat surprising, as one might expect the 15-25 and 26-40 year old age groups to increase over time because these are the age groups that are more likely to be injection drug users, and thus Hepatitis C positive.

The gender distribution of Hepatitis C patients is quite interesting. The proportion of males decreased from 84.6% in 1992 to 52% in 1995 and 1996, and then increased to 68.5% in 1999. On the other hand, the proportion of Hepatitis C positive females forms an opposite trend from 1992-99. See Figure 4.10 and Table 4.15. Perhaps the increase in the proportion of females over time in the whole cohort can be reflected by this relatively high proportion of females co-infected with HIV and HCV, as these patients that are co-infected are in fact a subset of the whole cohort.

4.4.3. Other Tables and Graphs.

The following graphs and their corresponding tables do not make use of one particular group of patients. Rather, these interesting findings use different groups of patients.

As would be expected, the distribution of Hepatitis C antibody status among non-IDUs for the whole cohort indicates that the majority of non-IDUs are not tested or negative for HCV. Only a very small proportion (4.2%) was positive for Hepatitis C. See Figure 4.11 and Table 4.16. This further confirms the fact that the majority of Hepatitis C positive patients are IDUs.

As indicated in Figure 4.12 and Table 4.17, Hepatitis C testing increased from1992, when 53.8% of IDUs were tested, to 1999, when 90.6% of IDUs were tested. As mentioned previously, prior to 1992 when HCV testing was not as sensitive and there was not a clearly defined and identifiable risk behaviour associated with Hepatitis C, many IDUs were not tested. Although the numbers are not shown in Figure 4.112 or Table 4.17, prior to 1992, less than 20% of IDUs were tested for HCV.

The distribution of risk behaviour in Hepatitis B surface antigen positive patients makes use of the whole cohort of patients. See Figure 4.13 and Table 4.18. The graph depicts a general decrease in the proportion of patients that are bisexual and homosexual, and an increase in the proportion of injection drug users from 1986-1999. Thus, it appears as though injection drug use is increasingly becoming a strong risk factor for acquiring Hepatitis B in this cohort of patients.

TABLE 4.1: DESCRIPTIVE ANALYSES OF SELECTED CHARACTERISTICS OF THE STUDY POPULATION

	Whole Cohort N=1,276	HIV+ Only N=950	HIV+ and HCV+ N=326
Gender, n (%)			
Male	1037 (81.3)	819 (86.2)	218 (66.9)
Female	239 (18.7)	131 (13.8)	108 (33.1)
Age[3], median (range)	32.8 (0-71.1)	32.9 (0-71.1)	32.7 (0.1-64.9)
Risk Behaviour, n (%)			
Bisexual, Homosexual	594 (46.6)	579 (60.9)	15 (4.6)
Heterosexual	184 (14.4)	175 (18.4)	9 (2.8)
Injection	430 (33.7)	140 (14.7)	290 (89.0)
Transfusion, Vertical	68 (5.3)	56 (5.9)	12 (3.7)
Baseline CD4 Count, mean (SD)	425.8 (337.5)	403.5 (340.7)	483.3 (322.8)
CD4 Cell Count[1], n (%)			
0-100	190 (14.9)	165 (17.4)	25 (7.7)
101-200	124 (9.7)	104 (10.9)	20 (6.1)
201-500	417 (32.7)	269 (28.3)	148 (45.4)
≥501	389 (30.5)	269 (28.3)	120 (36.8)
Missing	156 (12.2)	143 (15.1)	13 (4.0)
Baseline CD4%, mean (SD)	21.6 (12.1)	19.2 (11.6)	26.4 (11.6)
CD4 percent[1], n (%)			
0-8%	145 (11.4)	125 (13.2)	20 (6.1)
9-13%	112 (8.8)	92 (9.7)	20 (6.1)
14-28%	395 (31.0)	256 (26.9)	139 (42.6)
≥29%	260 (20.4)	134 (14.1)	126 (38.7)
Missing	364 (28.5)	343 (36.1)	21 (6.4)
HAART Treatment, n (%)			
Yes (HIV Viral Load ≤500)	374 (29.3)	250 (26.3)	124 (38.0)
No (HIV Viral Load >500)	264 (20.7)	125 (13.2)	139 (42.6)
Missing	638 (50.0)	575 (60.5)	63 (19.3)
Dead, n (%)			
Yes	346 (27.1)	319 (33.6)	27 (8.3)
No	930 (72.9)	631 (66.4)	299 (91.7)
Ethnic Status, n (%)			
Aboriginal	238 (18.7)	123 (12.9)	115 (35.3)
Caucasian	864 (67.7)	690 (72.6)	174 (53.4)
Other[2]	90 (7.1)	81 (8.5)	9 (2.8)
Blank, Unknown	84 (6.6)	56 (5.9)	28 (8.6)
Ever Jailed, n (%)			
Yes	140 (11.0)	31 (3.3)	109 (33.4)
No	1136 (89.0)	919 (96.7)	217 (66.6)
AIDS-defining illness			
Yes	475 (37.2)	425 (44.7)	50 (15.3)
No	801 (62.8)	525 (55.3)	276 (84.7)

[1] Categories are based on baseline CD4 cell count and baseline CD4 percent.

[2] Other corresponds to Black, Hispanic, Indian, and Oriental.

[3] Age is based on age when first seen.

TABLE 4.2: THE EFFECT OF HIV TREATMENT IN CO-INFECTED PATIENTS.

HIV endpoints	Effective Treatment		
	YES (viral load ≤500) N=76 (%)	NO (viral load >500) *N=250 (%)	Total *N=326(%)
Dead	3 (3.9)	24 (9.6)	27 (8.3)
Alive	73 (96.1)	226 (90.4)	299 (91.7)
AIDS-defining illness:			
YES	6 (7.9)	44 (17.6)	50 (15.3)
NO	70 (92.1)	206 (82.4)	276 (84.7)
CD4% ≤8%	7 (9.2)	36 (14.4)	43 (13.4)
CD4% >8%	69 (90.8)	208 (85.6)	277 (86.6)
CD4 count ≤100	6 (7.9)	41 (16.4)	47 (14.4)
CD4 count >100	70 (92.1)	209 (83.6)	279 (85.6)

*Sample sizes may not add up due to missing values.

TABLE 4.2.1: CHI-SQUARE TEST FOR THE EFFECT OF TREATMENT IN:

	Co-Infected Patients (N=326)	HIV Mono-Infected Patients (N=238)	HIV Positive Patients That Have Not Been Tested For HCV (N=182)
HIV Endpoint	P-value	P-value	P-value
Death	0.15	<0.001	<0.001
AIDS-Defining Illness	0.05	0.58	<0.001
CD4 Percent ≤8%	0.18	0.66	<0.001
CD4 Cell Count ≤100	0.09	0.58	<0.001

TABLE 4.3: THE EFFECT OF HIV TREATMENT IN HIV MONO-INFECTED PATIENTS.

HIV endpoints	Effective Treatment		Total N=238 (%)
	YES (Viral Load ≤500) N=100 (%)	NO (Viral Load >500) N=138 (%)	
Dead	4 (4.0)	33 (23.9)	37 (15.5)
Alive	96 (96.0)	105 (76.1)	201 (84.5)
AIDS-defining illness:			
YES	32 (32.0)	50 (36.2)	82 (34.5)
NO	68 (68.6)	88 (63.8)	156 (65.5)
CD4% ≤8%	30 (30.0)	37 (26.8)	67 (28.2)
CD4% >8%	70 (70.0)	101 (73.2)	171 (71.8)
CD4 count ≤100	32 (32.0)	49 (35.5)	81 (34.0)
CD4 count >100	68 (68.0)	89 (64.5)	157 (66.0)

TABLE 4.4: THE EFFECT OF HIV TREATMENT IN HIV POSITIVE PATIENTS THAT HAVE NOT BEEN TESTED FOR HCV.

HIV endpoints	Effective Treatment		Total *N=182(%)
	YES (viral load ≤500) *N=121 (%)	NO (Viral Load >500) *N=61 (%)	
Dead	4 (3.3)	13 (21.3)	17 (9.3)
Alive	117 (96.7)	48 (78.7)	165 (90.7)
AIDS-defining illness:			
YES	35 (28.9)	17 (27.9)	52 (28.6)
NO	86 (71.1)	44 (72.1)	130 (71.4)
CD4% ≤8%	47 (39.5)	19 (33.3)	66 (37.5)
CD4% >8%	72 (60.5)	38 (66.7)	110 (62.5)
CD4 count ≤100	42 (35.0)	18 (31.6)	60 (33.9)
CD4 count >100	78 (65.0)	39 (68.4)	117 (66.1)

*Sample sizes may not add up due to missing values.

TABLE 4.5: THE DISTRIBUTION OF HEPATITIS B SURFACE ANTIGEN AND ANTIBODY RESULTS.

	Whole cohort N=1,276	HIV+/HCV? N=950	HIV+/HCV- N=238	HIV+/HCV+ N=326
Hep B Vaccination, n(%)				
Yes	53 (4.2)	35 (3.7)	9 (3.8)	18 (5.5)
No	1223 (95.8)	915 (96.3)	229 (96.2)	308 (94.5)
Hep B surface antigen or antibody, n(%)				
Positive for Either	501 (39.3)	339 (35.7)	84 (35.3)	162 (49.7)
Negative for Both	568 (44.5)	434 (45.7)	142 (59.7)	134 (41.1)
Not Tested For Either	207 (16.2)	177 (18.6)	12 (5.0)	30 (9.2)

FIGURE 4.1: THE GENDER DISTRIBUTION IN THE WHOLE COHORT (1979-1999).

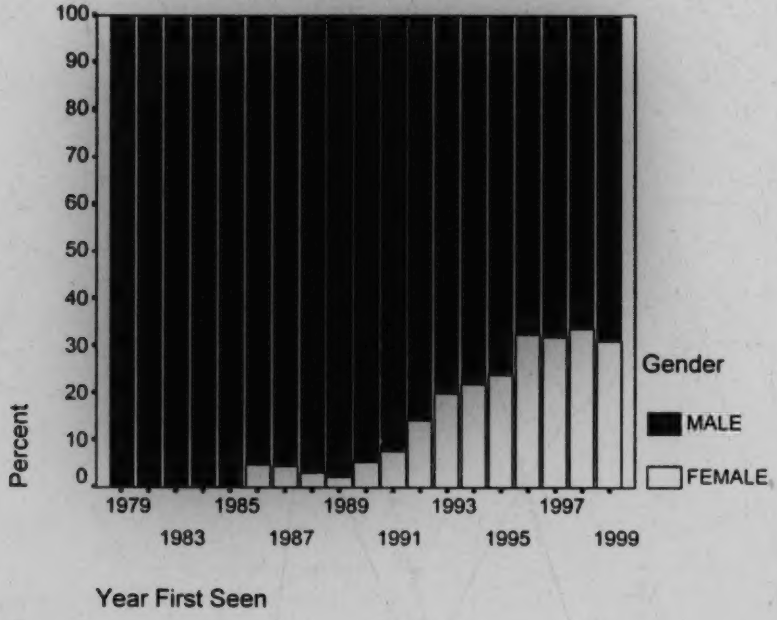


TABLE 4.6: THE GENDER DISTRIBUTION IN THE WHOLE COHORT (1979-1999)

	Year First Seen										
	1979	1980	1983	1984	1985	1986	1987	1988	1989	1990	1991
Female	0	0	0	0	0	2	3	2	2	5	7
Male	1	1	1	5	8	40	62	59	87	88	85
Total	1	1	1	5	8	42	65	61	89	93	92

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
Female	15	13	18	24	28	40	36	30	225
Male	91	52	64	76	58	85	71	66	1000
Total	106	65	82	100	86	125	107	96	1225

N=1,225 because of 51 missing values

FIGURE 4.2: THE DISTRIBUTION OF RISK BEHAVIOUR IN THE WHOLE COHORT (1979-1999).

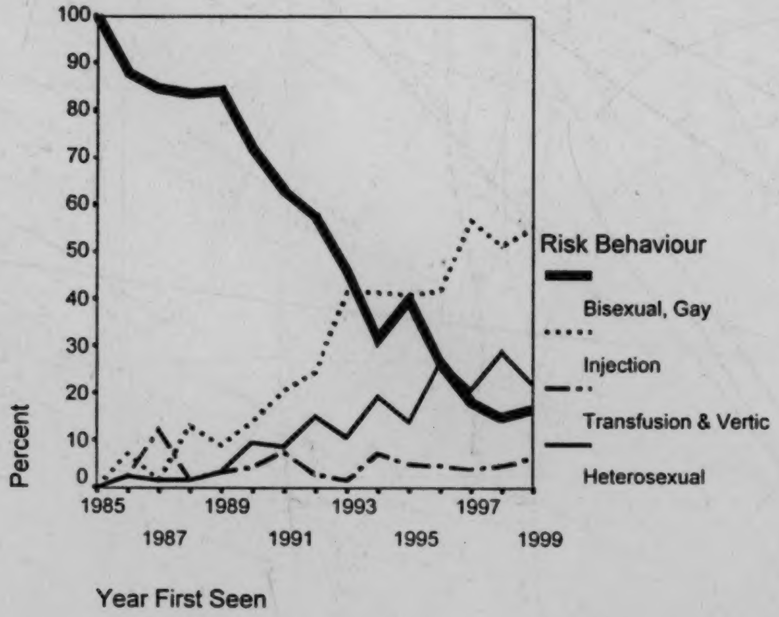


TABLE 4.7: THE DISTRIBUTION OF RISK BEHAVIOUR IN THE WHOLE COHORT (1979-1999).

	Year First Seen									
	1979	1980	1983	1984	1985	1986	1987	1988	1989	1990
Bisexual & Homosexual	0	1	1	3	8	37	55	51	75	67
Injection	0	0	0	0	0	3	1	8	8	13
Transfusion & Vertical	1	0	0	2	0	1	8	1	3	4
Heterosexual	0	0	0	0	0	1	1	1	3	9
Total	1	1	1	5	8	42	65	61	89	93

	Year First Seen									
	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total
Bisexual & Homosexual	58	61	30	26	40	23	23	16	16	591
Injection	19	26	27	34	41	36	71	55	53	395
Transfusion & Vertical	7	3	1	6	5	4	5	5	6	62
Heterosexual	8	16	7	16	14	23	26	31	21	177
Total	92	106	65	82	100	86	125	107	96	1225

N=1,225 because of 51 missing values.

FIGURE 4.3: THE DISTRIBUTION OF AGE GROUPS IN THE WHOLE COHORT (1979-1999).

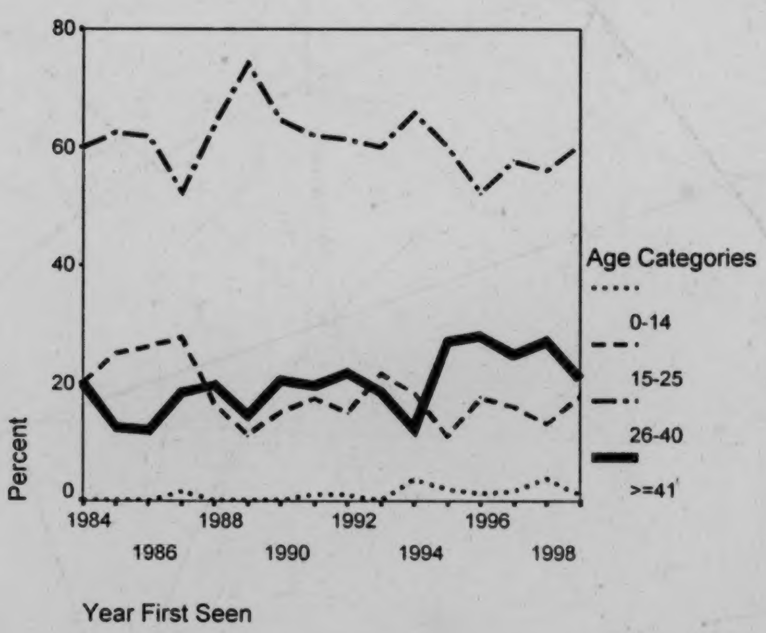


TABLE 4.8: THE DISTRIBUTION OF AGE GROUPS IN THE WHOLE COHORT, BASED ON AGE WHEN FIRST SEEN (1979-1999).

	Year First Seen									
	1979	1980	1983	1984	1985	1986	1987	1988	1989	1990
0-14	0	0	0	0	0	0	1	0	0	0
15-25	0	1	0	1	2	11	18	10	10	14
26-40	1	0	0	3	5	26	34	39	66	60
≥41	0	0	1	1	1	5	12	12	13	19
Total	1	1	1	5	8	42	65	61	89	93

	Year First Seen									
	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total
0-14	1	1	0	3	2	1	2	4	1	16
15-25	16	16	14	15	11	15	20	14	17	205
26-40	57	65	39	54	60	45	72	60	58	744
≥41	18	23	12	10	27	24	31	29	20	258
Total	92	106	65	82	100	86	125	107	96	1223

N=1,223 because of 53 missing values.

FIGURE 4.4: THE DISTRIBUTION OF HEPATITIS B ANTIGEN AND ANTIBODY STATUS IN THE WHOLE COHORT (1979-1999).

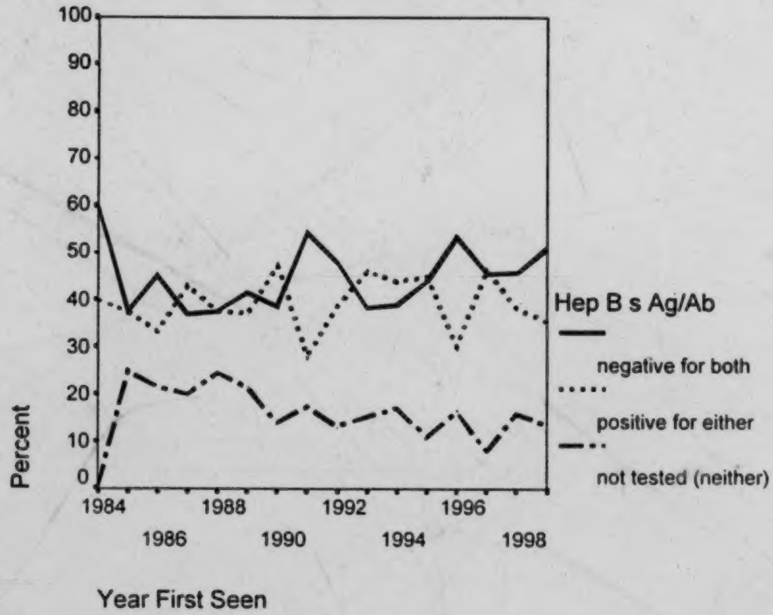


TABLE 4.9: THE DISTRIBUTION OF HEPATITIS B ANTIGEN AND ANTIBODY STATUS IN THE WHOLE COHORT (1979-1999).

	Year First Seen																			
	1979	1980	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total
Negative for both HBsAg & HBsAb	0	1	0	3	3	19	24	23	37	36	50	51	25	32	44	46	57	49	49	549
Positive for either HBsAg or HBsAb	0	0	1	2	3	14	28	23	33	44	26	41	30	36	45	26	58	41	34	485
Not tested for either HBsAg or HBsAb	1	0	0	0	2	9	13	15	19	13	16	14	10	14	11	14	10	17	14	191
Total	1	1	1	5	8	42	65	61	89	93	92	106	65	82	100	86	125	107	96	1225

N=1,225 because of 51 missing values.

FIGURE 4.5: THE DISTRIBUTION OF HEPATITIS C ANTIBODY RESULTS IN THE WHOLE COHORT (1992-1999).

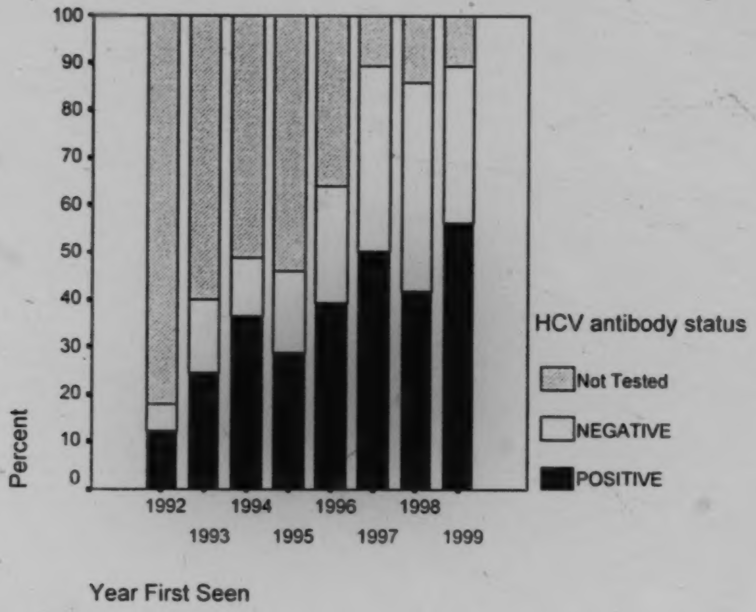


TABLE 4.10: THE DISTRIBUTION OF HEPATITIS C ANTIBODY RESULTS (1992-1999).

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
Hepatitis C Negative	6	10	10	17	21	49	47	32	192
Hepatitis C Positive	13	16	30	29	34	63	45	54	284
Not Tested	87	39	42	54	31	13	15	10	291
Total	106	65	82	100	86	125	107	96	767

FIGURE 4.6: THE DISTRIBUTION OF ETHNIC STATUS IN THE WHOLE COHORT (1979-1999).

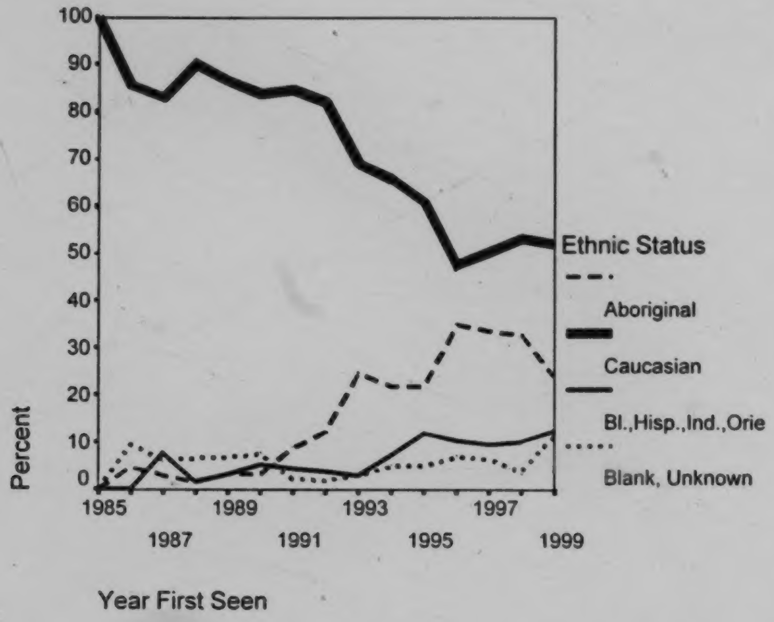


TABLE 4.11: THE DISTRIBUTION OF ETHNIC STATUS IN THE WHOLE COHORT (1979-1999).

	Year First Seen									
	1979	1980	1983	1984	1985	1986	1987	1988	1989	1990
Aboriginal	0	0	0	0	0	2	2	1	3	3
Caucasian	1	1	1	5	8	36	54	55	77	78
Black,Hispanic Indian,Oriental	0	0	0	0	0	0	5	1	3	5
Blank, Unknown	0	0	0	0	0	4	4	4	6	7
Total	1	1	1	5	8	42	65	61	89	93

	Year First Seen									
	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total
Aboriginal	8	13	16	18	22	30	42	35	23	218
Caucasian	78	87	45	54	61	41	63	57	50	852
Black, Hispanic, Indian, Oriental	4	4	2	6	12	9	12	11	12	86
Blank, Unknown	2	2	2	4	5	6	8	4	11	69
Total	92	106	65	82	100	86	125	107	96	1225

N=1,225 because of 51 missing values.

FIGURE 4.7: THE DISTRIBUTION OF ETHNIC STATUS IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

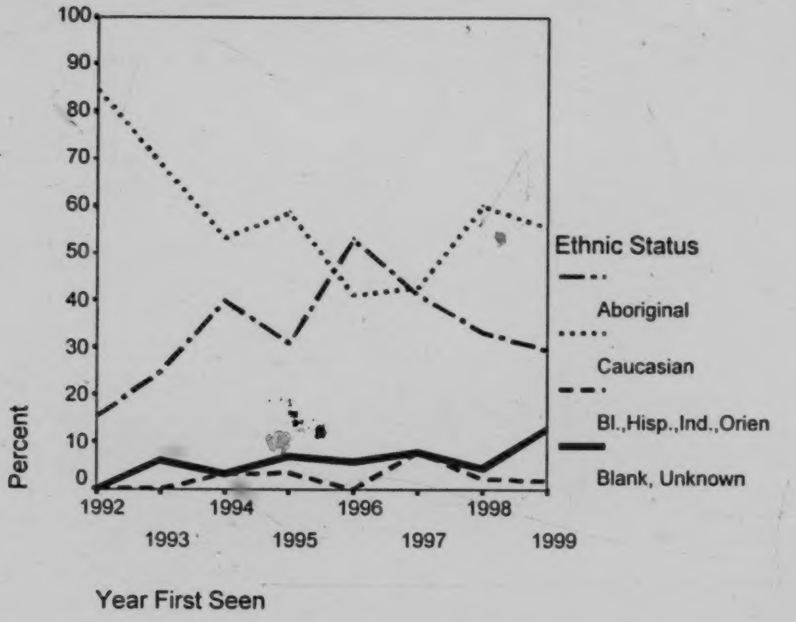


TABLE 4.12: THE DISTRIBUTION OF ETHNIC STATUS IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
Aboriginal	2	4	12	9	18	26	15	16	102
Caucasian	11	11	16	17	14	27	27	30	153
Black, Hispanic, Indian, Oriental	0	0	1	1	0	5	1	1	9
Blank, Unknown	0	1	1	2	2	5	2	7	20
Total	13	16	30	29	34	63	45	54	284

FIGURE 4.8: THE DISTRIBUTION OF RISK BEHAVIOUR IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

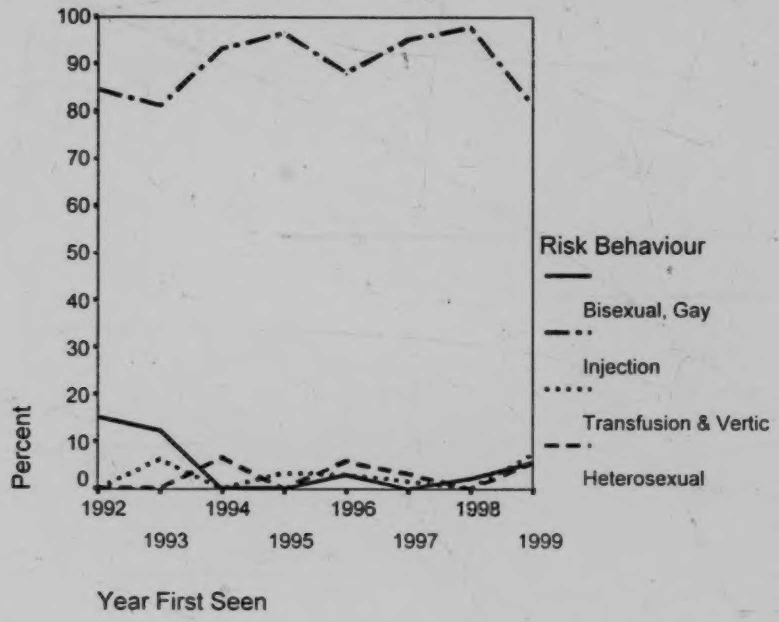


TABLE 4.13: THE DISTRIBUTION OF RISK BEHAVIOUR IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
Bisexual & Homosexual	2	2	0	0	1	0	1	3	9
Injection	11	13	28	28	30	60	44	44	258
Transfusion & Vertical	0	1	0	1	1	1	0	4	8
Heterosexual	0	0	2	0	2	2	0	3	9
Total	13	16	30	29	34	63	45	54	284

FIGURE 4.8A: THE DISTRIBUTION OF RISK BEHAVIOUR IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

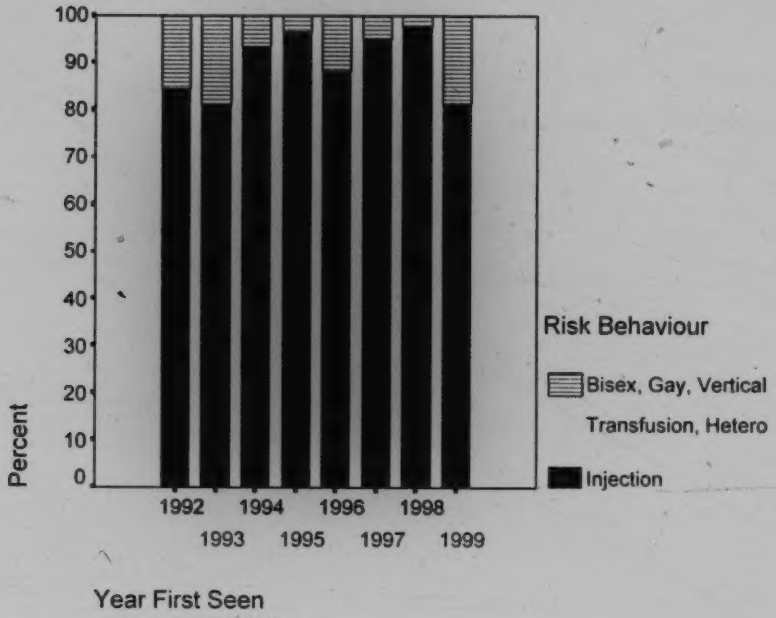


TABLE 4.13A: THE DISTRIBUTION OF RISK BEHAVIOUR IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
Bisexual, Homosexual, Heterosexual, Vertical, Transfusion	2	3	2	1	4	3	1	10	26
Injection	11	13	28	28	30	60	44	44	258
Total	13	16	30	29	34	63	45	54	284

FIGURE 4.9: THE DISTRIBUTION OF AGE GROUPS IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

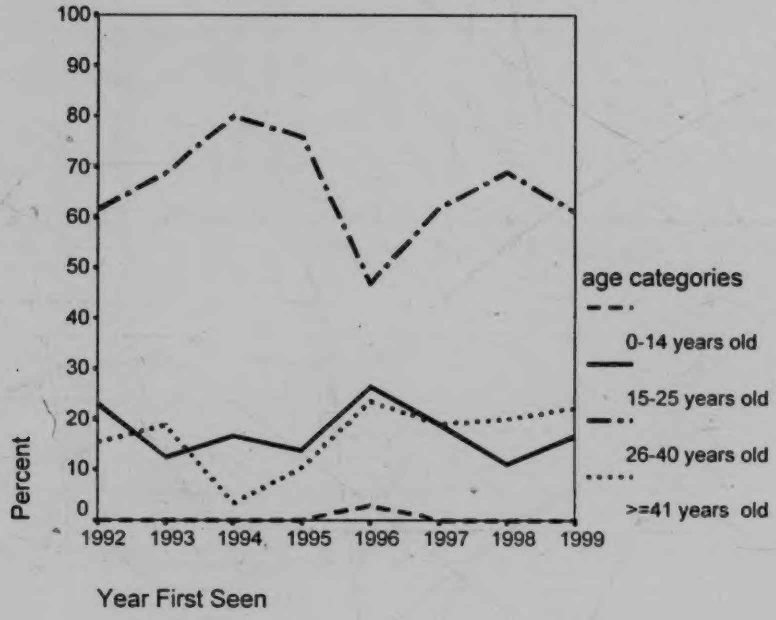


TABLE 4.14: THE DISTRIBUTION OF AGE GROUPS (BASED ON THE AGE WHEN FIRST SEEN) IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
0-14	0	0	0	0	1	0	0	0	1
15-25	3	2	5	4	9	12	5	9	49
26-40	8	11	24	22	16	39	31	33	184
≥41	2	3	1	3	8	12	9	12	50
Total	13	16	30	29	34	63	45	54	284

FIGURE 4.10: THE GENDER DISTRIBUTION IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

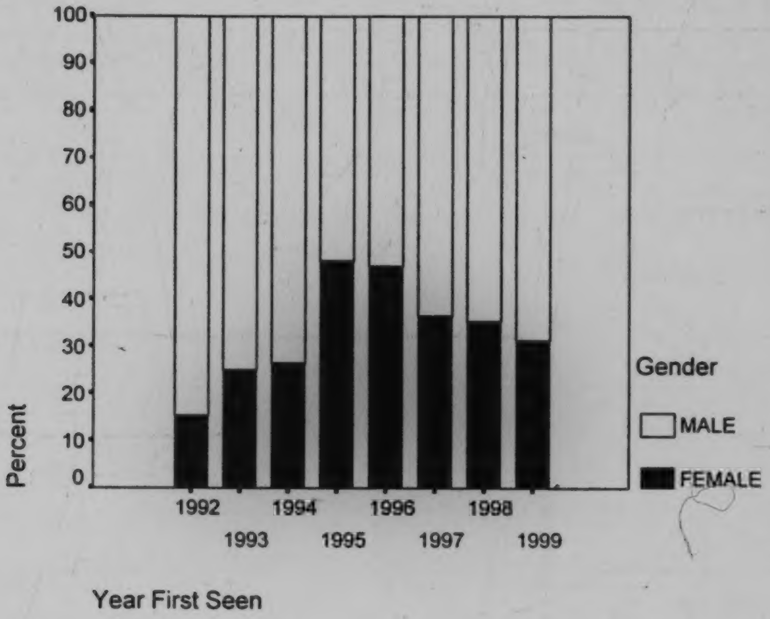


TABLE 4.15: THE GENDER DISTRIBUTION IN HEPATITIS C POSITIVE PATIENTS (1992-1999).

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
Female	2	4	8	14	16	23	16	17	100
Male	11	12	22	15	18	40	29	37	184
Total	13	16	30	29	34	63	45	54	284

FIGURE 4.11: THE DISTRIBUTION OF HEPATITIS C ANTIBODY STATUS IN NONN-INJECTION DRUG USERS IN THE WHOLE COHORT (1979-1999).

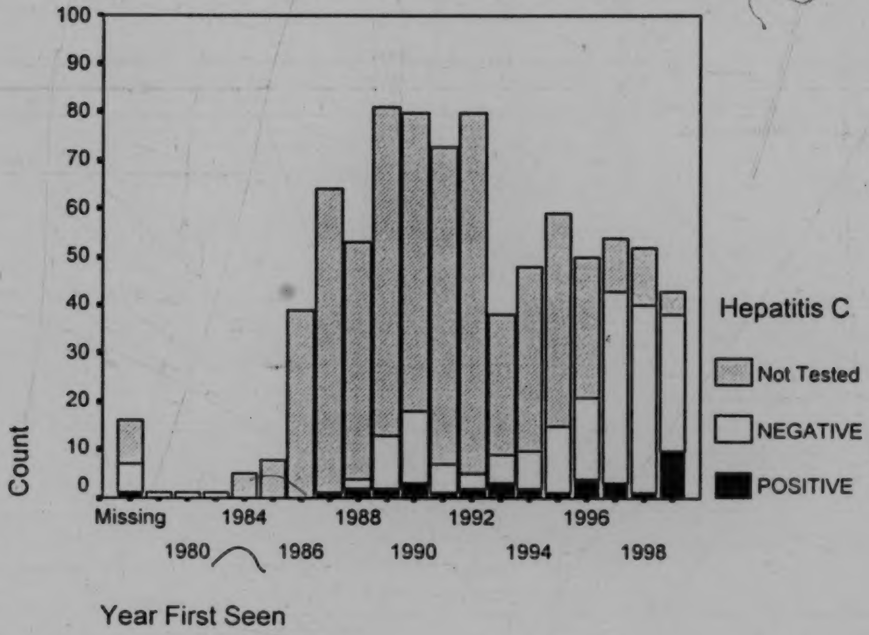


TABLE 4.16: THE DISTRIBUTION OF HEPATITIS C ANTIBODY STATUS IN NON-INJECTION DRUG USERS IN THE WHOLE COHORT (1979-1999).

	Year First Seen									
	1979	1980	1983	1984	1985	1986	1987	1988	1989	1990
Hep C Positive	0	0	0	0	0	0	1	2	2	3
Hep C Negative	0	0	0	0	0	0	0	2	11	15
Not Tested	1	1	1	5	8	39	63	49	68	62
Total	1	1	1	5	8	39	64	53	81	80

	Year First Seen									
	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total
Hep C Positive	1	2	3	2	1	4	3	1	10	35
Hep C Negative	6	3	6	8	14	17	40	39	28	189
Not Tested	66	75	29	38	44	29	11	12	5	606
Total	73	80	38	48	59	50	54	52	43	830

N=830 because of 51 missing values and 395 IDUs.

FIGURE 4.12: THE DISTRIBUTION OF HEPAITTIS C ANTIBODY STATUS IN INJECTION DRUG USERS (1992-1999).

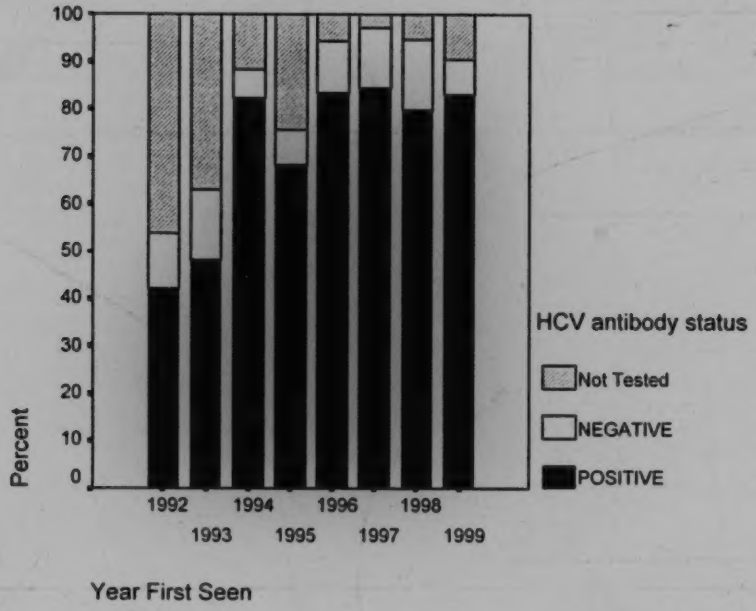


TABLE 4.17: THE DISTRIBUTION OF HEPATITIS C ANTIBODY STATUS IN INJECTION DRUG USERS (1992-1999).

	Year First Seen								
	1992	1993	1994	1995	1996	1997	1998	1999	Total
Hepatitis C Negative	3	4	2	3	4	9	8	4	37
Hepatitis C Positive	11	13	28	28	30	60	44	44	258
Not Tested	12	10	4	10	2	2	3	5	48
Total	26	27	34	41	36	71	55	53	343

FIGURE 4.13: THE DISTRIBUTION OF RISK BEHAVIOUR IN HEPATITIS B SURFACE ANTIGEN POSITIVE PATIENTS FOR THE WHOLE COHORT (1986-1999).

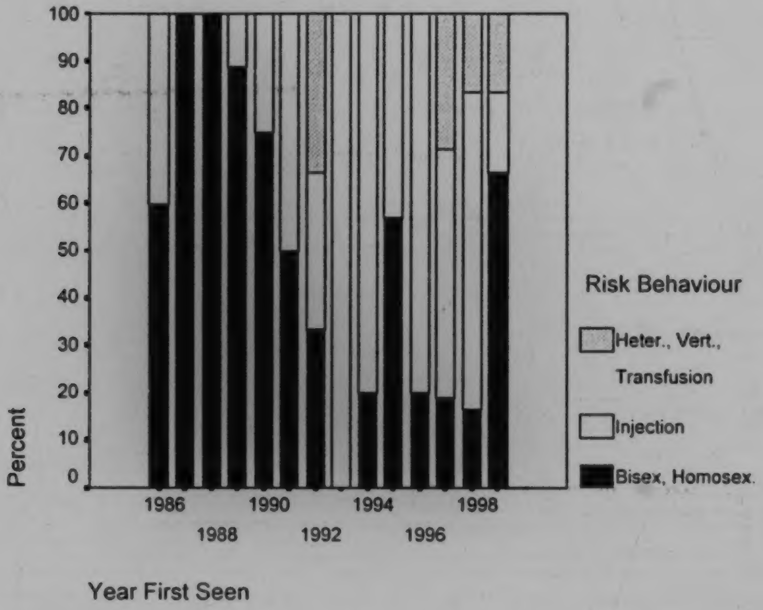


TABLE 4.18: THE DISTRIBUTION OF RISK BEHAVIOUR IN HEPATITIS B SURFACE ANTIGEN POSITIVE PATIENTS FOR THE WHOLE COHORT (1986-1999).

	Year First Seen									
	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
Bisexual, Homosexual	3	8	6	8	6	2	2	0	1	4
Injection	2	0	0	1	2	2	2	3	4	3
Heterosexual, Transfusion & Vertical	0	0	0	0	0	0	2	0	0	0
Total	5	8	6	9	8	4	6	3	5	7

	Year First Seen				
	1996	1997	1998	1999	Total
Bisexual, Homosexual	1	4	1	4	50
Injection	4	11	4	1	39
Heterosexual, Transfusion & Vertical	0	6	1	1	10
Total	5	21	6	6	99

CHAPTER FIVE

RESULTS – KAPLAN-MEIER CURVES AND SURVIVAL ANALYSES

This chapter focuses exclusively on the Kaplan-Meier (KM) curves, which were performed prior to any Cox regression analyses. The first set of KM curves relates Hepatitis C status to the HIV endpoint of a CD4 count ≤ 100 using: 1) all survival times, 2) minimum survival of 24 months, 3) minimum survival of 36 months, 4) minimum survival of 48 months, and 5) minimum survival of 60 months. This allows the reader to visualize and clearly understand why the inclusion criteria for all KM curves and survival analyses from this point onwards require that patients survive a minimum of 48 months. The second set of KM curves relates Hepatitis C status to each of the other three HIV endpoints (CD4 percent $\leq 8\%$, AIDS-defining illness, and death), using the inclusion criteria of a minimum survival of 48 months.

5.1 Introduction to Kaplan-Meier Curves and the Log-Rank Test

Survival curves are estimated and graphed using the Kaplan-Meier (KM) method. In performing survival analyses, there is always an outcome of interest, and (survival) time is measured until an event or censoring occurs. An event is often also referred to as a "failure". For the purposes of this study, there are four outcome variables of interest, all of which are HIV endpoints: 1) survival time (months) until CD4 count ≤ 100, 2) survival time (months) until CD4 percent $\leq 8\%$, 3) survival time (months) until an AIDS-defining illness, and 4) survival time (months) until death. The HIV endpoints are precisely the events.

KM survival curves are a plot of the estimated survival probabilities corresponding to each failure time. Survival probabilities are the probability that a patient will survive past a specified time. The survival probability is as follows [64]:

$$S(t) = \Pr(T > t)$$

where T is the survival time of the random variable, and t is the specific value of T.

The log-rank test is the most commonly used test to compare two or more survival curves. The log-rank test is approximately chi-square test with G-1 degrees of freedom that makes use of observed versus expected cell counts over categories of outcomes, thereby providing an overall comparison of the KM curves. When calculating the degrees of freedom, G equals the number of groups being compared. The null hypothesis for the log-rank statistic is that there is no overall difference between the survival curves. The log-rank statistic is as follows [64]:

Log-rank statistic = $(O_i - E_i)^2 / \text{Var}\,(O_i - E_i)$

where "O" is the number of observed counts, "*i*" is the number of groups being compared, and "E" is the number of expected counts. "E" is the product of the proportion in the risk set and the number of failures over both groups.

5.2 The Effect of a Time Bias in the Cox Regression Analysis

Recall that for the purposes of all survival analyses the cohort was first limited to those patients who have been tested for Hepatitis C. This gave a sample size of 564: 326 whom are Hepatitis C positive and 238 whom are Hepatitis C negative. Thereafter, the cohort was further limited to patients who had been first seen from 1992 onwards; this effectively minimized any biases that may have been introduced by earlier Hepatitis C testing, as mentioned in the methods section of this paper. Therefore, the final sample size for the first set of survival analyses was 476.

Survival times were computed in the same manner for all four HIV endpoints. If the patient did not reach the endpoint by the end of follow-up for the study, their survival time was defined as the end date (December 31, 1999) minus the date they were seropositive for HIV. On the other hand, if the patient did reach one of the selected HIV endpoints, their survival time was computed as time interval corresponding to the date when the endpoint was achieved minus the date the patient became seropositive for HIV.

All survival times were in months. The entire cohort has been followed to a maximum of 180 months. It is important to note, however, that Figures 5.1 through 5.6 have been truncated on the right, for convenience, after the last patient reached an endpoint. Once the survival times had been calculated and defined for each of the four HIV endpoints, and entered into the calculation of the KM curves, the following results appeared.

Upon carrying out the first set of KM curves, it became evident that there was a very surprising pattern occurring with the data, as can be seen in Figure 5.1. That is, the KM curves were showing that patients who were co-infected with HIV and Hepatitis C had a better survival than patients only infected with HIV. Although Kaplan-Meier survival curves were created for all four HIV endpoints, only the Kaplan-Meier curve for the HIV endpoint CD4 count ≤100 is shown (Figure 5.1). All four of the graphs, each depicting a different HIV endpoint, gave similar results. Clearly, this was a counterintuitive finding, as we would expect co-infected patients to have a worse survival.

5.2.1 Patients Who Progressed To An HIV Endpoint

A closer examination of the data led to the discovery that a large number of the survival times were either very short or negative. The reason for these very short and sometimes negative survival times can be attributed to a number of factors. Although there are only a handful of negative survival times for each of the four HIV endpoints (less than 10 for each endpoint), it may be indicative of a more systematic problem. Firstly, patients who had the negative or short survival times were primarily patients who had achieved the HIV endpoint. The explanation for these patients' short or negative survival times is probably a function of their lifestyle. Many of these patients lead very chaotic lifestyles. It is suspected that these individuals may not have known they were infected with HIV for a long period of time. Also, a significant minority of homosexual men presented with a diagnosis of HIV when they had already progressed to one of the endpoints; these patients either misjudged their risk or were in denial, both normal human behaviours. These individuals are the types that would not be expected to regularly seek medical care. In any event, by the time they had sought medical care, they were suspected to have had HIV already for several years. Because these patients would not seek regular medical attention and it is suspected that they had been infected with HIV for a long period of time prior to their diagnosis, it is very possible that the HIV endpoint may have been achieved either in advance of the HIV diagnosis or shortly thereafter. Therefore, these patients' HIV-related illness had likely already progressed to an endpoint by the time they were diagnosed with HIV, or their HIV was so far along that it wasn't long before an endpoint was achieved.

5.2.2 Patients Who Did Not Progress To An HIV Endpoint

There is a different explanation for those patients who did not achieve an HIV endpoint, but still managed to have very short survival times. Because these patients do not have the HIV endpoint, their survival time is simply their end of follow-up date (December 31, 1999) minus their date they were seropositive for HIV. Therefore, these patients have short survival times simply because they are recent entrants into the database. In addition, some of these patients may have died of non-AIDS-defining causes; examples of these endpoints include motor vehicle accidents and suicide. The majority of the patients with short survival times were diagnosed as seropositive for HIV and entered into the database during the year 1999. Insofar as could be examined, these patients' short survival times could not be attributed to any underlying factors. It is important to note that these patients did not have negative survival times; it was only the patients who had achieved the HIV endpoint that accounted for the negative survival times.

5.3 The Selection of 48 Months for the Remainder of the Survival Analyses.

Upon defining the survival times and carrying out the KM analyses, the above findings with the time bias in the database, gave very counterintuitive results. Therefore, Kaplan-Meier analyses were performed at time intervals, for all four HIV endpoints, conditional on survival times to at least 24 months, 36 months, 48 months, and 60 months, respectively. The natural course of HIV disease progression to any of the four chosen endpoints can be up to 10 years in patients not receiving HIV treatment. Therefore, by requiring a minimum survival of 24, 36, 48, and 60 months for successive KM analyses, it was possible to examine how the survival curves changed as a result of successively limiting the analysis to increasing survival times. By doing this, it was anticipated that the majority of patients who presented late in the course of their illness would be eliminated.

These KM figures showing the effects of a time bias are only shown for the HIV endpoint of a CD4 count ≤100, as the other three endpoints illustrated the same results. These figures can be compared to the initial Kaplan-Meier curve (Figure 5.1), where all survival times were used in the analysis. As can be seen in Figures 5.2 through 5.5, as longer survival times were selected, the figures began to depict something very different than the original figure (Figure 5.1) when all survival times were incorporated. Recall that originally, when all survival times were used, HIV and HCV co-infected patients appeared to have a better survival then those only infected with HIV. However, even when survival times were only limited to being at least 24 months, the survival curves were flipping and what appeared to emerge was that patients who are co-infected, in fact, had a worse survival probability as compared to patients who were only infected with HIV.

It was decided that, for the purposes of this study, all survival analyses from this point forward would limit the data to survival times having at least 48 months, regardless of the HIV endpoint under consideration. Therefore, all Kaplan-Meier curves and Cox regression analyses from this point onward made use of data that had a minimum survival time of 48 months. In this manner, the data was more reliable; limiting the survival analyses to a minimum survival of 48 months eliminated not only the negative survival times that had been encountered earlier, but the majority of the short survival times would be excluded as well. As previously mentioned, although the graphs for the CD4 count endpoint are the only ones depicted here, the other three HIV endpoints had identical results. That is, when all survival times were used, it appeared as though co-infected patients had a better survival probability than patients only infected with HIV. Thus, the analyses performed with these other three endpoints also only made use of survival times that had a minimum survival of 48 months.

The log-rank statistic for the Kaplan-Meier curve in Figure 5.4, which used a minimum of 48 months survival has a p-value of 0.51. While Figure 5.4 indicates that patients co-infected with HIV and HCV appear to have a worse survival probability for the HIV endpoint of a CD4 count ≤100, the finding is not statistically significant. This finding is astonishingly different from that when all survival times were used (Figure 5.1). In this figure, the log-rank statistic yielded a p-value <0.001. Thus, when all survival times were used, patients who were co-infected with Hepatitis C had a statistically significant better survival probability than patients who were only infected with HIV. See Table 5.1 for the log-rank statistic for each survival time for the HIV endpoint CD4 count ≤100.

5.3.1 The Effect of Limiting the Survival Analyses to a Minimum of 48 Months Survival.

As a result of excluding cases from the initial survival cohort (n=476), when all survival times were used, the sample size was reduced by a considerable amount. By the time the survival cohort had been limited to survival times that were at least 48 months (for the HIV endpoint of a CD4 count ≤100), only 161 patients were included for the analysis. The purpose for requiring a minimum of 48 months survival is because we have a significant number of inaccurate, or strictly speaking, unknown dates for HIV seroconversion. Moreover, a number of patients entered the database when they were much further along in the course of their infection than others, and there was no way of sorting these patients out. Finally, there is likely a systematic bias, as co-infected patients are likely to be more recently infected. By eliminating those who had very little time left at the point of entry in to the database, there were 161 patients remaining for the analyses. This is a very small sample size, which makes it difficult to detect a statistically significant difference in survival between the two groups. In addition, we lose the ability to analyze the survival pattern of those patients who did not survive a minimum of 48 months.

5.4 Kaplan-Meier Curves Stratified by Hepatitis C for All Four HIV Endpoints, Using a Minimum of 48 Months Survival.

Once the time bias had been established and corrected for, Kaplan-Meier curves were performed for the other three HIV endpoints, using a minimum survival of 48 months.

See Figures 5.6 through 5.8. Refer to Figure 5.4 for the Kaplan-Meier curve illustrating survival until CD4 count ≤100.

Although the two curves cross in Figure 5.6, up until 84 months survival it seems as though patients who are co-infected with Hepatitis C have a worse survival probability for the HIV endpoint of a CD4 percent ≤8%. However, from the log-rank statistic, this finding is not significant (p=0.47), as indicated in Table 5.2. It is interesting to note that after 84 months survival, there are no patients that achieve the HIV endpoint of a CD4 percent ≤8% in the co-infected group, whereas in the group infected only with HIV, only 3 patients reached this HIV endpoint. Thus the fact that the two curves cross at 84 months is probably negligible, as only a very small number of events occurred in the HIV-infected group.

The Kaplan-Meier survival curve relating Hepatitis C status to the HIV endpoint AIDS-defining illness indicates that those co-infected with Hepatitis C appear to have a worse survival prognosis, as revealed by the lower survival curve for co-infected patients. See Figure 5.7. Once again, this finding is not significant (p=0.72), which is evident, as there is no obvious separation between the two survival curves.

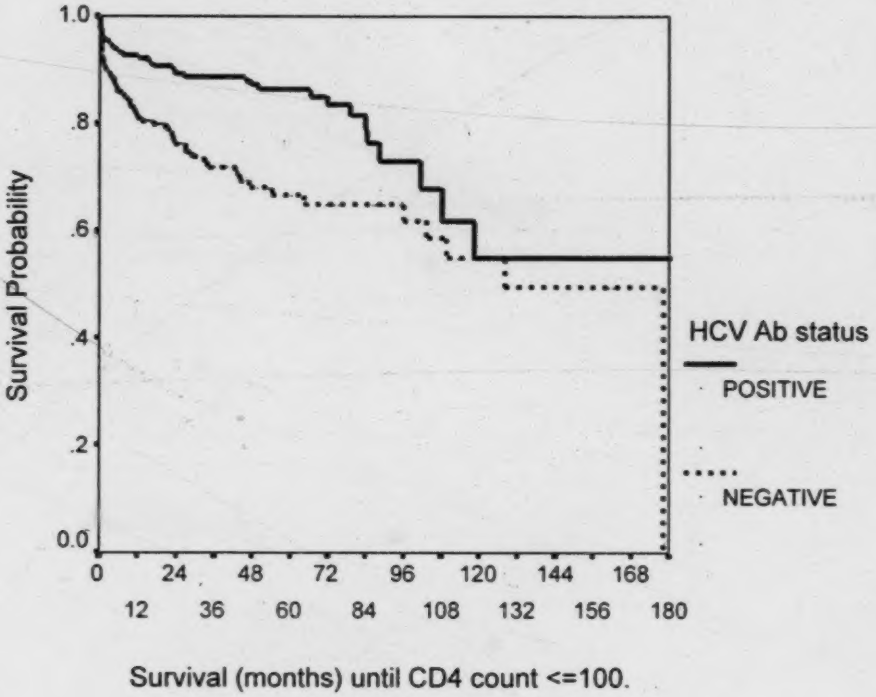
The last survival curve (Figure 5.8) illustrates that co-infected patients seem to have a worse prognosis for the HIV endpoint death. Therefore, patients co-infected with Hepatitis C progress more rapidly to death. However, as indicated by the log-rank statistic this outcome is not statistically significant (p=0.60).

5.5 Conclusions

There are four HIV endpoints that were selected for the purposes of the survival analyses. These are: 1) CD4 cell count ≤100, 2) CD4 percent ≤8%, 3) AIDS-defining illness, and 4) death. Once the time bias in the data had been controlled for by excluding patients that did not survive a minimum of 48 months, KM survival curves were performed relating each of the four HIV endpoints to Hepatitis C status.

For all of the four HIV endpoints chosen for the survival analysis, the group of patients co-infected with Hepatitis C had a worse survival prognosis than the group of patients only infected with HIV. Nevertheless, none of these findings were statistically significant, as indicated by the log-rank statistic and it's associated p-value.

FIGURE 5.1: KAPLAN-MEIER SURVIVAL CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT CD4 COUNT ≤100, USING ALL SURVIVAL TIMES.



NOTE: The entire cohort has been followed to a maximum of 180 months. Figures 5.1 through 5.6 have been truncated on the right (for convenience) after the last patient reached an HIV endpoint. Thus, follow-up is at most from 1985. The explanation that a few HIV events occurred after 96-months can be accounted for by the fact that HIV serostatus was known, in some cases, prior to the date of first registration (i.e., date first seen) in the database. Survival times have been calculated using the date of known HIV seropositivity as the start time, whereas, the selection of patients from 1992 onwards was made using the date on which a patient was first seen. In most cases, the date of known HIV seropositivity and the date a patient was first seen are identical or very close (perhaps one or two weeks apart). However, in some cases, the date of known HIV seropositivity occurred a lot earlier than the date a patient was first seen. For example, some patients from outside of Northern Alberta may have been diagnosed as HIV seropositive for some time prior to moving to Northern Alberta.

FIGURE 5.2: KAPLAN-MEIER SURVIVAL CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT CD4 COUNT ≤100, CONDITIONAL ON SURVIVAL TO 24 MONTHS.

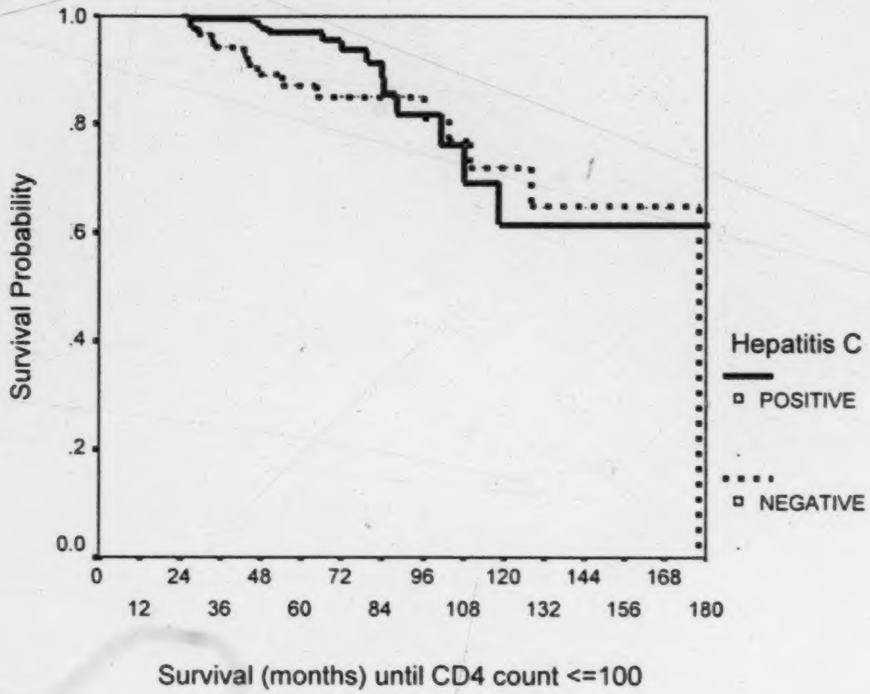


FIGURE 5.3: KAPLAN-MEIER SURVIVAL CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT CD4 COUNT ≤100, CONDITIONAL ON SURVIVAL TO 36 MONTHS.

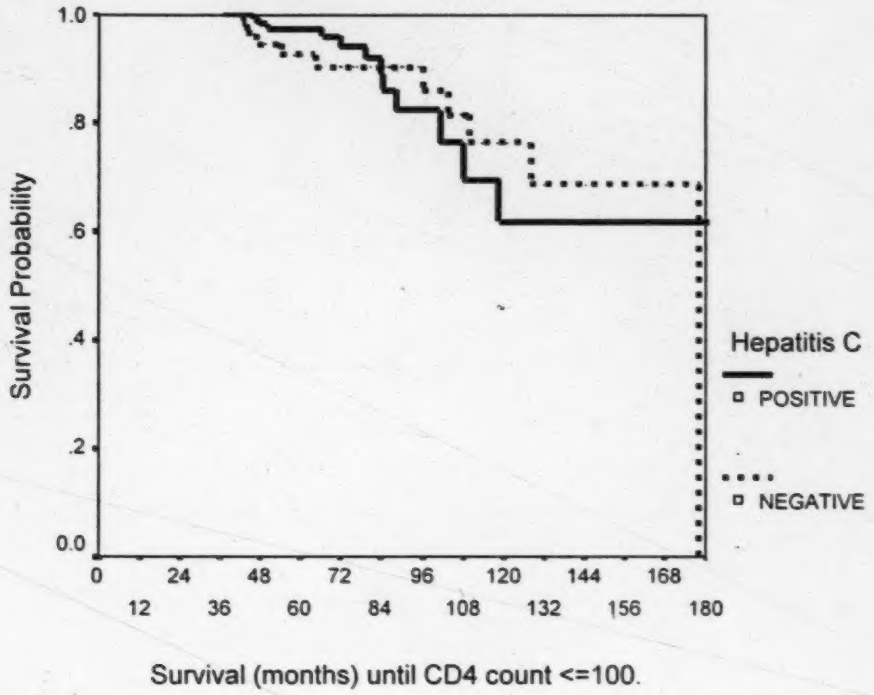


FIGURE 5.4: KAPLAN-MEIER SURVIVAL CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT CD4 COUNT ≤100, CONDITIONAL ON SURVIVAL TO 48 MONTHS.

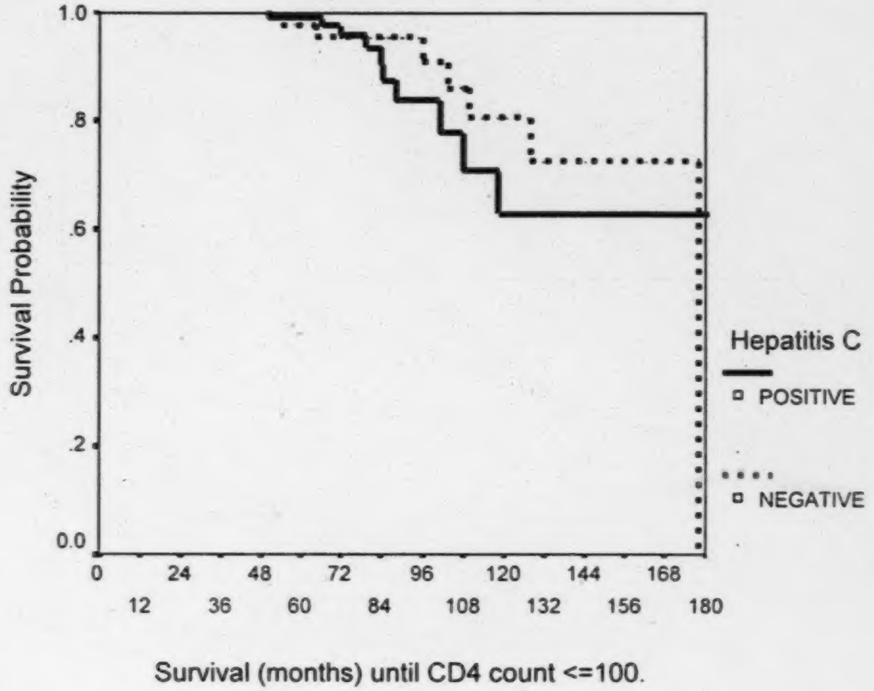


FIGURE 5.5: KAPLAN-MEIER SURVIVAL CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT CD4 COUNT ≤100, CONDITIONAL ON SURVIVAL TO 60 MONTHS.

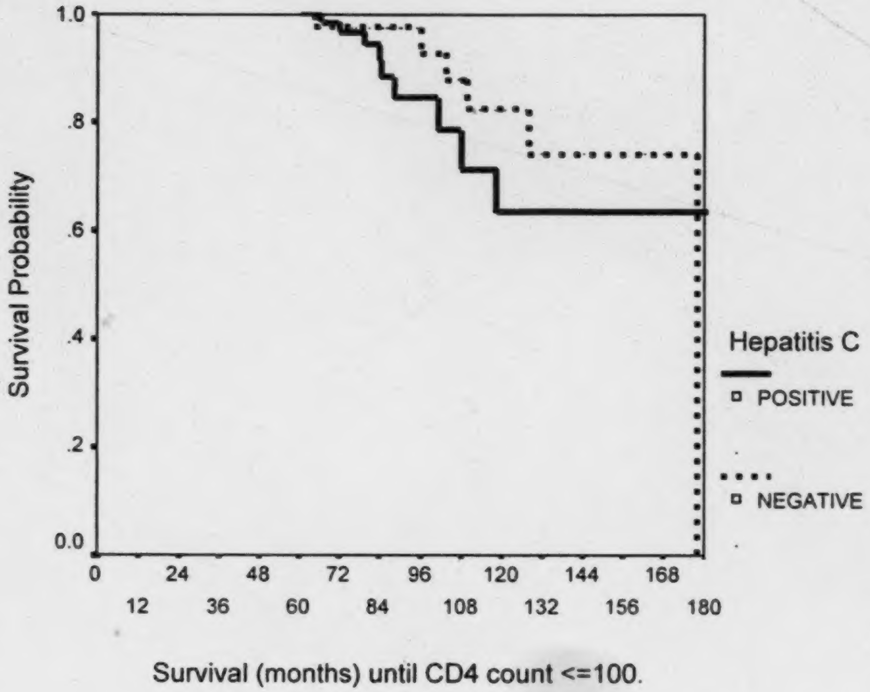


TABLE 5.1: LOG-RANK STATISTIC FOR EACH SURVIVAL TIME FOR THE HIV ENDPOINT CD4 COUNT ≤100.

Survival Time	Log-Rank	df	P-value	Number of patients
All	13.61	1	<0.001	460
≥24 Months	1.44	1	0.23	291
≥36 Months	0.00	1	0.96	213
≥48 Months	0.44	1	0.51	161
≥60 Months	0.80	1	0.37	125

FIGURE 5.6: KAPLAN-MEIER CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT CD4 PERCENT ≤ 8%, CONDITIONAL ON SURVIVAL TO 48 MONTHS.

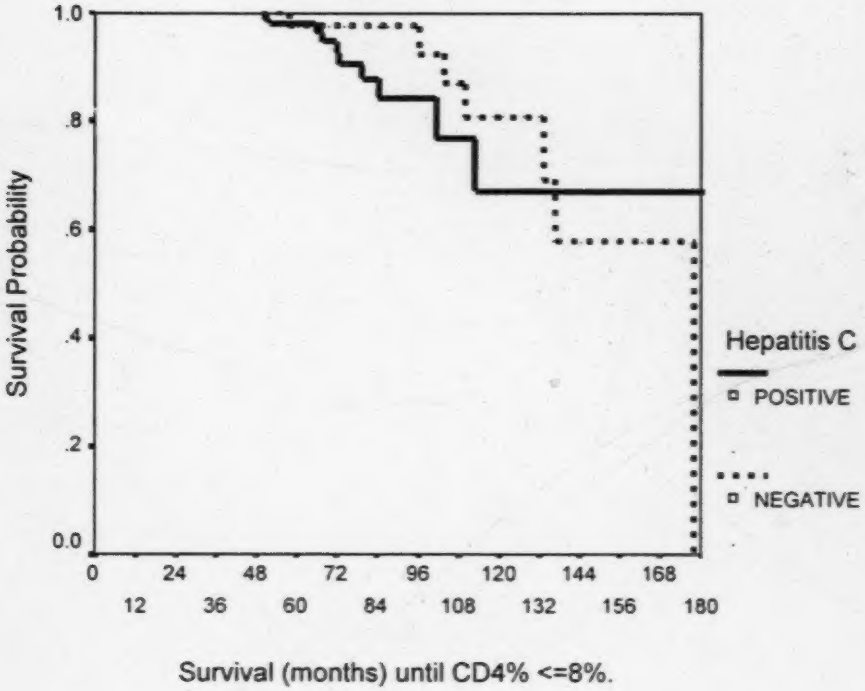


FIGURE 5.7: KAPLAN-MEIER CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT AIDS, CONDITIONAL ON SURVIVAL TO 48 MONTHS.

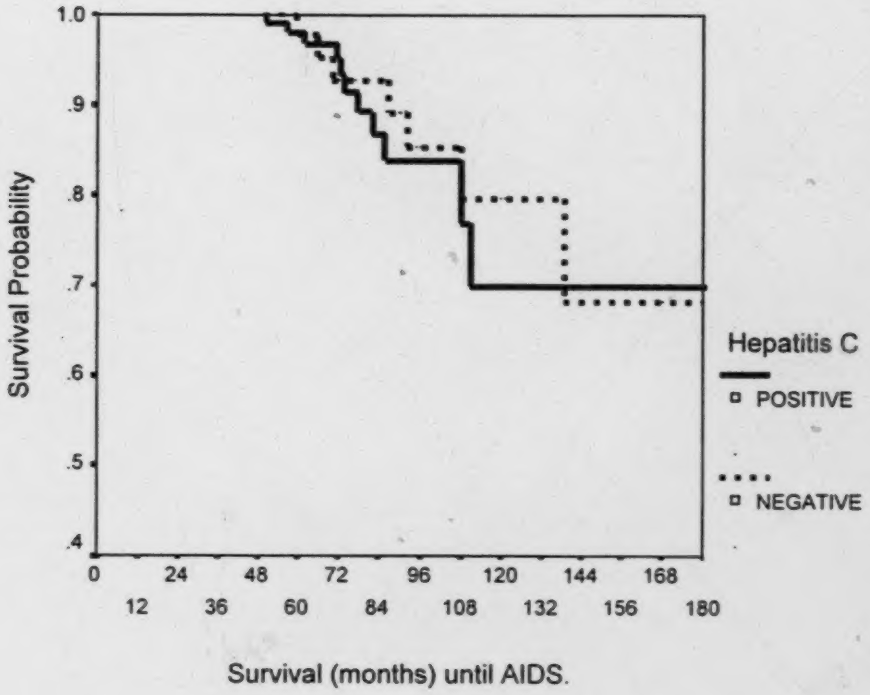


FIGURE 5.8: KAPLAN-MEIER CURVE RELATING HEPATITIS C STATUS TO THE HIV ENDPOINT DEATH, CONDITIONAL ON SURVIVAL TO 48 MONTHS.

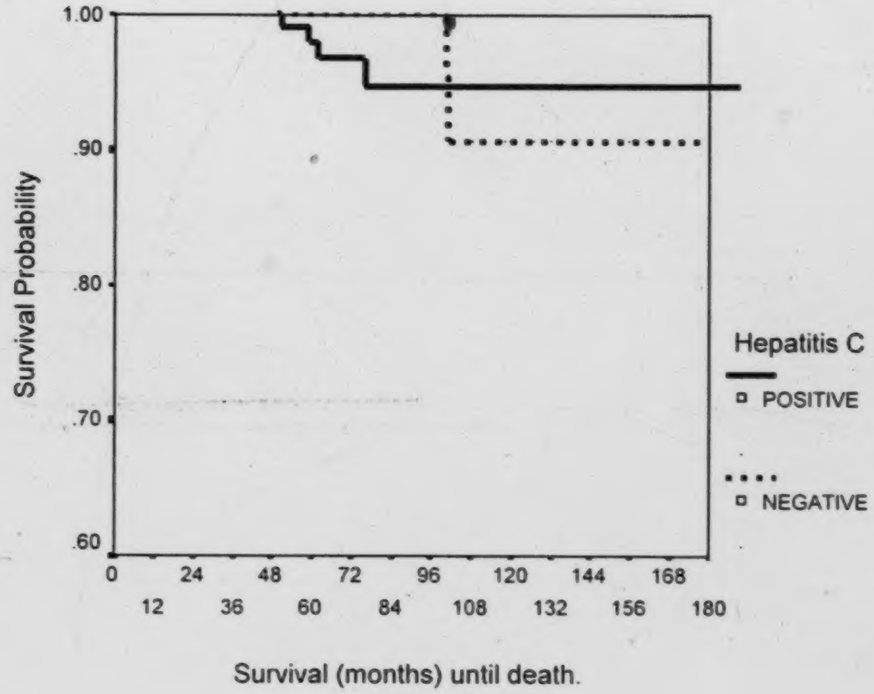


TABLE 5.2: LOG-RANK STATISTIC FROM THE KAPLAN-MEIER CURVE FOR EACH OF THE FOUR HIV ENDPOINTS, USING A MINIMUM OF 48 MONTHS SURVIVAL.

HIV Endpoint	Log-Rank	df	P-value	Number in cohort	Number of events
1) CD4 Count ≤100	0.44	1	0.51	160	17
2) CD4 Percent ≤8%	0.53	1	0.47	154	17
3) AIDS-defining illness	0.12	1	0.72	163	18
4) Death	0.28	1	0.60	173	6

NOTE: The number in the cohort varies from N=154 to N=173 due to missing values.

CHAPTER SIX

RESULTS – COX REGRESSION SURVIVAL ANALYSIS

The Cox proportional hazards (PH) model is a widely used mathematical model used for analyzing survival data. The objective of this model is to compare the survival experience of two groups, while adjusting for possible confounding and interaction effects. Furthermore, this analysis is meant to identify and examine potential risk factors and determinants associated with progression to each of the four HIV endpoints. The Cox regression survival analysis was chosen owing to the "time to event" nature of the four outcome variables. Recall that the outcome variables are the four HIV endpoints.

6.1 Introduction to the Cox Model.

The Cox model is usually written in terms of the hazard model formula.

$$h(t,X) = h_0(t) \exp\left(\sum_{i=1}^{p} \beta_i X_i\right)$$

This equation is an expression of the hazard at time t for an individual with a given set of explanatory variables, which are denoted by the X_i's. Thus, the X_i's represents a set of predictor variables that are being modeled to predict an individual's hazard [64]. This formula states that the hazard at time t is the product of the baseline hazard function, $h_0(t)$, and the following expression,

$$\exp\left(\sum_{i=1}^{p} \beta_i X_i\right)$$

The baseline hazard function, $h_0(t)$, is the hazard function obtained when no explanatory variables are considered in the model. The baseline hazard is an unspecified function, and it is this property that makes the Cox model a nonparametric model. Even though, the baseline hazard is unspecified, the β_i's can still be estimated. As well, the hazard ratio is estimated without having to estimate the baseline hazard function.

The hazard ratio (HR) is the measure of effect obtained in a survival analysis. It is the hazard for one individual divided by the hazard for a different individual. The two individuals being compared are distinguished by their values for a set of predictor variables. Let X_i^* represent the set of predictors for one individual and X_i represent the set of predictors for another individual. Then the hazard ratio comparing the individuals is:

$$\mathrm{HR} = \exp\left[\sum_{i=1}^{p} \beta_i (X_i^* - X_i)\right]$$

The interpretation of the HR is similar to that of an odds ratio. For example, a hazard ratio of 1 means that there is no effect; therefore, 1 is the null value for the exposure-outcome relationship. A hazard ratio greater than 1 is interpreted as the exposed group having an increased hazard over the unexposed group, by a factor equal to the hazard ratio. Conversely, a hazard ratio less than 1 is interpreted as the exposed group having a decreased hazard over the unexposed group, by a factor equal to the hazard ratio. There is a relationship between the survival probability, obtained from the Kaplan Meier curves, and the hazard rate; the higher the survival probability at time *t*, the lower is the corresponding hazard rate, and vice versa [64].

For the Cox model, SPSS output includes: 1) the variable(s) that have been included in the model, 2) the regression coefficients corresponding to each variable in the model, which are represented by β, 3) the standard errors (SE) of the regression coefficients, 4) the p-values for testing the significance of each coefficient, 5) the hazard ratio (HR), which is a point estimate for the effect of each variable adjusted for the other variables in the model, 6) the confidence interval for the effect, and 7) the P(PH) which allows us to evaluate the proportional hazards assumption [64].

The method of estimation that is used to obtain the coefficients for the Cox regression model is the maximum likelihood (ML) method. There are two test statistics typically used with the maximum likelihood (ML) estimates. The first of the two test statistics is the Wald statistic. This is the test statistic used in this study. The other statistic is the likelihood ratio (LR) statistic, which makes use of the log likelihood value. While the LR and Wald statistics may not give exactly the same answer, the LR statistic has better statistical properties. Therefore, the LR test should be used [64].

The P(PH) provided in the output allows one to evaluate the proportional hazard (PH) assumption for each variable in the model. The value given is a p-value, which is derived from a standard normal statistic. A nonsignificant, or large, p-value ($p>0.10$) means the PH assumption has been satisfied. On the other hand, a significant, or small, p-value ($p<0.05$) indicates that the variable being tested has not satisfied the PH assumption.

6.2 The Meaning of the Proportional Hazard Assumption

The proportional hazard (PH) assumption requires that the hazard ratio (HR) is constant over time, or equivalently, that the hazard for one individual is proportional to the hazard for any other individual, where the proportionality constant is independent of time [64].

This assumption of the PH model means that the explanatory or predictor variables do not involve *t*; that is, they are time-independent. Time-independent variables are values for an individual that do not change over time. Some examples include gender and ethnic status. An individual's risk behaviour may change over time, but for the purposes of this study and analysis, variables such as risk behaviour are assumed not to change once they have been measured.

6.3 Cox Regression with Hepatitis C as the Only Main Effect for Each of the Four HIV Endpoints.

In the first group of analyses, only Hepatitis C status was entered as a predictor variable for each of the four HIV endpoints. Hepatitis C status was the only predictor variable entered because initially, we wanted to determine the effect of this variable on each of the four HIV endpoints, as this is the main focus of the study. As established in Chapter 5, survival times were included only if they were a minimum of 48 months, thus effectively reducing the aforementioned time bias. Table 6.1 provides the results of the Cox regression for the predictor variable Hepatitis C and each of the four HIV endpoints.

The sample size for the Cox models where Hepatitis C was entered as the single main effect varied from N=156 to N=177 because of missing values and depending on which HIV endpoint was being considered. Because the HIV endpoint CD4 count ≤100 (N=161) is used for the remainder of the Cox analyses, this is the sample size specified in the methods section in Chapter 3.

From Table 6.1, it is evident that Hepatitis C does not have a large measure of effect on any of the four HIV endpoints. The measure of effect of Hepatitis C varies from 1.2 to 1.6. While these measures of effect for Hepatitis C are not large by usual epidemiologic standards, they are of potential clinical significance. A hazard of 1.4 in the Cox analysis relating Hepatitis C status to the HIV endpoint of a CD4 count ≤100 means that patients who are Hepatitis C positive have a 40% increased risk of progressing to this HIV endpoint than do patients who are only infected with HIV. This should not be viewed as a small biological effect, as a 40% increased risk is, in fact, quite substantial. Moreover, a hazard ratio of 1.6 for the HIV endpoint death means patients who are Hepatitis C positive have a 60% increased risk of progressing to death than patients who are mono-infected with HIV if, in fact, the difference is real. Similar interpretation can be made with the HIV endpoints CD4 percent and AIDS-defining illness.

While the hazard ratios can be viewed as clinically significant, the Wald p-values are large, indicating that the effect of Hepatitis C on each of the four HIV endpoints is not statistically significant. These large p-values are likely a reflection of the small sample size. Therefore, the problem is not that of small measures of effect, rather it is with respect to the lack of statistical significance, which can largely be attributed to a small sample size.

Hepatitis C had very similar effects on both survival until CD4 count ≤100 and survival until CD4 percent ≤8%. As previously indicated, a hazard ratio of 1.4 for CD4 count

≤100 means that patients who are co-infected with Hepatitis C and HIV have 1.4 times the hazard of achieving a CD4 count ≤100 than patients only infected with HIV. The same interpretation applies for the HIV endpoint CD4 percent, which also yielded a hazard of 1.4. However, neither of the measures of effect for Hepatitis C status relating survival to these two HIV endpoints was statistically significant. From Chapter five, Figures 5.4 and 5.6 are the Kaplan-Meier curves for Hepatitis C and survival until CD4 count ≤100 and survival until CD4 percent ≤8%, respectively. In both figures, the curves only cross over minimally.

If we go back to the Kaplan-Meier curve in Figure 5.7, we notice that the two survival curves cross substantially. Because there is no clear separation between the two survival curves, we cannot view Hepatitis C as predictive of reaching the HIV endpoint of AIDS. Moreover, this HIV endpoint revealed the smallest measure of effect, as indicated in Table 6.1 (HR=1.2). Moreover, onset of AIDS is considered to be an increasingly poor endpoint as prophylaxis and highly active antiretroviral drugs improve the outcomes of HIV-infected patients. For these reasons, the HIV endpoint of an AIDS-defining illness will no longer be used in any further Cox model survival analyses. Recall that the focus of this study is to determine whether Hepatitis C is predictive in reaching any of the above stated HIV endpoints.

The Cox regression with survival until the HIV endpoint of death revealed the largest effect, which was 1.6. However, the confidence interval for this hazard ratio was very wide (0.3-9.0), thus making the point estimate imprecise. Similar to the endpoint of an AIDS-defining illness, death is increasingly considered a weak endpoint with the advent of HAART in late 1996. While the long-term benefit of these therapies is yet to be determined, it has become apparent that fewer people are reaching an AIDS endpoint or a death endpoint related to HIV/AIDS. As Table 5.2 illustrates, there were very few events that occurred in either group of patients. In fact, between both groups, only 6 events occurred making it difficult to analyze them any further. As a result, the HIV endpoint of death will no longer be used in any further survival analyses.

The Cox models from this point onwards will focus solely on the HIV endpoint of CD4 count ≤100. There is no particular reason why the HIV endpoint of a CD4 count ≤100 was chosen over the HIV endpoint of a CD4 percent ≤8%. They are just different measures of the same biologic phenomenon. Both revealed very similar measures of effect when Hepatitis C status was entered into the model, and their Kaplan-Meier curves were very similar for Hepatitis C status as well. It is important that the endpoint chosen for any further analyses satisfy the proportional hazard assumption when Hepatitis C is entered as a predictor variable, as this is the main variable for which we are trying to obtain a measure of effect.

In order to determine whether the PH was satisfied, log-log plots were performed for the predictor variable Hepatitis C, for all four HIV endpoints. In addition, log-log plots were performed for all predictor variables that were entered into the Cox models for the HIV endpoint CD4 count ≤100. All log-log plots satisfied the proportional hazard assumption, and therefore the predictor variables can be considered time-independent. In this manner, the HRs can be viewed as constant over time, or equivalently, that the hazard for one individual is proportional to the hazard for any other individual, where the proportionality constant is independent of time

6.4 Variable Selection for Cox Analyses With One Main Effect

All variables considered in the Cox analyses with one main effect are listed in Table 6.2 with their associated hazard ratios. These analyses are crude models, as they do not consider the effect of potential covariates of interest. Most of the variables selected for the analyses are dichotomous. However, those variables that were not already dichotomous were collapsed into two biologically meaningful categories.

The variable risk behaviour was dichotomized as either having a history of injection drug use (IDU) or not having a history of IDU. Risk behaviour was dichotomized in this manner, as a large majority of co-infected patients are IDUs. Interestingly, the hazard ratio was protective, meaning that patients with a history of IDU had a decreased hazard of 50% of reaching the HIV endpoint of a CD4 count ≤100 (HR=0.5). More often in the literature, patients with a history of IDU have a worse outcome, as they lead very chaotic lifestyles and are often very inconsistent in adhering to a drug regimen. The protective effect may be attributed to the younger age of these patients. Therefore, an interaction term of age and risk behaviour was entered into the Cox model, but it was not statistically significant, indicating there is no significant interaction between age and risk behaviour.

The variable gender yielded a hazard ratio of 1.0, which is the null value, indicating that there is no increased hazard for men reaching the HIV endpoint. Another surprising finding was when the variable "ever jailed" was entered into the analysis. Patients who had been previously jailed had a decreased hazard of obtaining the HIV endpoint by a factor of 0.4. This is surprising as it is expected that patients who had been incarcerated at some point in their lives would have an increased hazard of achieving the HIV endpoint. Patients who have been jailed are often transient and chaotic individuals, all characteristics which are often associated with poor health outcomes.

Baseline CD4 count was dichotomized as having a baseline CD4 count ≤500 or ≥501, and baseline CD4 percent was dichotomized as having a baseline CD4 percent of ≥29% or ≤28%. Patients who had a baseline CD4 count ≤500 had an increased hazard of reaching the HIV endpoint by a factor of 2.0. Moreover, patients who had a baseline CD4 percent ≤28% had an increased hazard of reaching the HIV endpoint by a factor of 2.3. However, these findings were not statistically significant.

The variable of ethnicity was dichotomized twice; the first dichotomization was either Aboriginal ancestry or other, and the second dichotomization was as Caucasian ancestry or other. The first dichotomization revealed that Aboriginals have 1.1 times the hazard of obtaining the HIV endpoint of a CD4 count ≤100 compared to all others. Conversely, Caucasians revealed a protective measure of effect; Caucasians had a decreased hazard of achieving the endpoint compared to all others, by a factor of 0.8. However, neither of the findings with respect to ethnicity was statistically significant. Age category was dichotomized into 0-25 year olds and ≥26 years old. The Cox analyses with age category entered as the single main effect indicated that the group of patients ≥26 years old had 2.8

times the hazard of achieving the HIV endpoint than patients 25 or younger. This is congruent with what is found in the literature; however, the result was not statistically significant.

Patients who were positive for one or both of Hepatitis B surface antigen or antibody had a null effect on obtaining the HV endpoint; the measure of effect was 1.0 with a corresponding p-value of 0.99. Therefore, this variable was not predictive at all. As would be expected, patients who have had an AIDS-defining illness had an increased hazard (HR=6.3) of reaching a CD4 count ≤100; this finding was highly statistically significant ($p<0.001$). Finally, patients who have not responded well to HIV treatment (that is, they have a viral load measurement >500) have an increased hazard of reaching the HIV endpoint by a factor of 2.7; this finding nearly attained significance, with a p-value of 0.08.

Because there were very few predictor variables that yielded significant results, the focus was placed on the hazard ratio estimates from this point onwards, as opposed to p-values. It is most likely that the lack of significant results obtained in the Cox analyses with one main effect is likely the result of a small sample size. Sample sizes varied from 115 to 160 depending on which variable was entered into the Cox regression analysis (there were some missing values for each predictor variable). The fact that the majority of the hazard ratios for the predictor variables in the Cox regression analyses, with one main effect, hovered around the null value of 1 may be a reflection of the small sample size. However, it may also indicate that a lack of effect; in order to determine whether this is the case, a larger sample size is required.

6.5 Cox Regression Analyses With Two Main Effects for the HIV Endpoint CD4 Count ≤100.

The variables considered for the Cox analyses with two main effects were chosen by examining the above Cox analyses that used a single main effect. All analyses with two main effects contain as covariates Hepatitis C and one other variable.

6.6 Confounding

Confounding involves the possibility that an observed association may be due to the effects of differences between study groups, other than the exposure under study that could risk their outcome of developing the outcome of interest [65]. As such, confounding is a function of the complex interrelationships between various exposures and disease. In order for a variable to be a confounder, it must be associated with the exposure of interest, and independent of the exposure, also be a risk factor for the disease or outcome of interest. Confounding may bring about an overestimate or underestimate of the true association between an exposure and outcome, and may even result in a change in direction of the observed effect.

6.6.1 Confounding of Other Predictor Variables

In this study, Hepatitis C status represents the exposure variable of primary interest, and the outcome is the HIV endpoint of a CD4 count ≤100. All other predictor variables that were entered into the Cox models with two main effects were considered as potential confounders of Hepatitis C. It is important to note that, while a confounder must be predictive of the disease or outcome of interest, the association does not have to be causal. Often, confounding variables are only correlates of another causal factor. For example, age and gender are associated with virtually all diseases and are related to the presence or level of a number of exposures. For this reason, they should always be considered as potential confounders of an association. These variables, however, may not be causally related to the disease of interest but rather act as surrogates for etiologic factors [65]. Thus, the goal here is to describe the effect of Hepatitis C status adjusted for each of the other predictor variables, when each was added to a model with two main effects. By comparing the hazard ratios for Hepatitis C in Table 6.2 and Table 6.3, we can determine whether there is any confounding effect by any of the other predictor variables on the association between Hepatitis C status and the HIV endpoint of a CD4 count ≤100.

When Hepatitis C was entered into the crude analysis, the associated hazard ratio was 1.4 and the p-value was 0.5. We can compare this hazard ratio to those obtained in Table 6.3, when Hepatitis C status was entered into a Cox regression along with one other predictor variable. The hazard ratio for Hepatitis C changed to 1.9 when risk behaviour was entered as a covariate into the analysis. The estimated hazard ratio obtained for Hepatitis C is somewhat higher in the analysis where risk behaviour is controlled for than the corresponding value obtained in the crude model (HR=1.4). In this model, patients who are Hepatitis C positive have a 90% increased hazard of progressing to this HIV endpoint than do patients who are only infected with HIV. In addition, the hazard ratio for Hepatitis C changed to 1.8 when AIDS-defining illness was entered as a covariate into the analysis. Again, this estimated hazard ratio obtained for Hepatitis C is somewhat higher than that obtained in the Cox model where Hepatitis C was the single main effect (HR=1.4). With this Cox model, Hepatitis C positive patients have an 80% increased risk of reaching this HIV endpoint than do patients who are only infected with HIV. Recall that with Hepatitis C entered as the single main effect in the Cox model, Hepatitis C positive patients have a 40% increased hazard of reaching the HIV endpoint of a CD4 count ≤100. When other covariates were entered into the model alongside Hepatitis C, the hazard ratio did not change much from the value of 1.4, which was the hazard ratio calculated for Hepatitis C in the crude model. Thus, we can conclude that there is confounding due to the predictor variables risk behaviour and AIDS-defining illness on the association between Hepatitis C status and the HIV endpoint of a CD4 count ≤100, because the crude versus adjusted hazard ratios for Hepatitis C are meaningfully different. It is somewhat surprising that the predictor variables, baseline CD4 cell count and baseline CD4 percent, were not confounders for the relationship between Hepatitis C and HIV, because, intuitively, we would expect that his might be the case.

The fact that the variables AIDS-defining illness and risk behaviour were found to have confounding effects on the relationship between Hepatitis C status and the HIV endpoint of a CD4 count ≤100 is indicative that these two variables are associated with Hepatitis C status and, independent of Hepatitis C status, predictive of the HIV endpoint. The relationship between a confounder and an outcome are not always clear. Risk behaviour is associated with Hepatitis C status in that a majority of Hepatitis C positive patients are IDUs. With respect to risk behaviour, it may be postulated that some risk groups progress more quickly to an HIV endpoint than do others. Recall that risk behaviour is dichotomized into IDUs and non-IDUs, and in the Cox model with risk behaviour as the single main effect the hazard ratio was protective for IDUs (HR=0.5). It is evident that IDU behaviour, while protective in a model when it is the single main effect, increased the effect of Hepatitis C status on reaching the HIV endpoint from 40% to 90% in the Cox model with these two main effects. Therefore, in this case risk behaviour as a confounder lead to an underestimate of the true association between Hepatitis C and the HIV endpoint.

With regards to the confounder AIDS-defining illness, there is an obvious biological association between this predictor variable and the HIV endpoint of a CD4 count ≤100. Clearly, patients who have an AIDS-defining illness are more likely to progress to this endpoint. The CD4 count will vary depending on what AIDS-defining illness a patient is inflicted with. Furthermore, some patients may have more than one AIDS-defining illness, thereby lowering their CD4 count further AIDS-defining illness is considered to be a negative confounder because it lead to an underestimate of the measure of effect.

6.6.2 Confounding of Hepatitis C

If Hepatitis C is a confounder for any of the predictor variables entered into the Cox analyses with two main effects, the crude hazard ratio for the selected predictor variable as the single main effect will be meaningfully different from the hazard ratio obtained when the predictor variable was entered together with Hepatitis C status. Thus, in these analyses, it is Hepatitis C status that is being controlled for.

The hazard ratio for risk behaviour jumped from 2.1 in the crude model to 2.7 when Hepatitis C was added in the Cox model with two main effects. There was a moderate change in the hazard ratio for baseline CD4 percent when Hepatitis C status was added to the model; the hazard ratio increased from 2.3 up to 2.7. The only other variable for which the hazard ratio changed when Hepatitis C was added is for AIDS-defining illness. The crude HR increased from 6.3 to 6.9 when Hepatitis C was added to the model. Therefore, we can conclude that there is confounding with respect to Hepatitis C status for the variables risk behaviour, baseline CD4 percent, and AIDS-defining illness, because the crude versus adjusted HRs for these predictor variables are meaningfully different.

6.7 Cox Regression Analyses With Three or More Main Effects for the HIV Endpoint CD4 Count ≤100.

Upon examining the results from the previous models with one and two main effects, it was decided that the following predictor variables needed to be examined in Cox models with three or more main effects.

Table 6.4 has three models, each consisting of three or more main effects. Each model includes Hepatitis C status as one of the main effects. Model 1 assessed the predictor variables Hepatitis C, risk behaviour, and AIDS-defining illness. The results of the regression analysis reveal a HR of 2.5 for patients that are Hepatitis C positive. This value nearly attained statistical significance (p=0.09). Therefore, patients co-infected with Hepatitis C have 2.5 times the hazard of reaching a CD4 count ≤100, when Hepatitis C status is entered alongside risk behaviour and AIDS-defining illness in the Cox model. This HR is much higher than that obtained when Hepatitis C status was entered as the single main effect in the Cox model (HR=1.4). Thus, it can be concluded that risk behaviour category and AIDS-defining illness are negative confounders for Hepatitis C.

Interestingly, in Model 1, the risk behaviour of IDU continued to behave in a protective manner for progression to a CD4 count ≤100. The HR of 0.3 was significant (p=0.02); note that this HR was reduced further (i.e. it has become more protective) from when risk behaviour was entered as the single main effect (HR=0.5). The fact that we continue to find IDU to be protective of progression to the HIV endpoint of CD4 count ≤100, may be attributed to the younger age of these patients. IDU is generally associated with younger age. However, there was no significant interaction between age and IDU behaviour. Furthermore, while AIDS-defining illness reached statistical significance with a HR of 8.3, the CI was very wide. This is largely due to the small sample size. In any event, as would be expected, patients who have or have had an AIDS-defining illness have 8 times the hazard of reaching a CD4 count ≤100.

Model 2 included Hepatitis C status, AIDS-defining illness, and HIV treatment. From Table 6.4, AIDS-defining illness and HIV treatment attained statistical significance. Thus, patients who have an AIDS-defining illness or have not had effective HIV treatment have an increased hazard of reaching a CD4 count ≤100 by 9.9 and 3.3, respectively. Hepatitis C positive patients have an increased hazard of reaching a CD4 count ≤100 by 1.9; however, this finding was not statistically significant (p=0.27). Thus, not only did the HR for Hepatitis C decrease from Model 1, but the findings were even less statistically significant.

In the final Cox model, Model 3, Hepatitis C status, risk behaviour, AIDS-defining illness, and HIV treatment were entered. The HR for Hepatitis C was 2.2, but it did not reach statistical significance (p=0.16). The HR for risk behaviour decreased further to a value of 0.2 (p=0.01). Finally, while AIDS-defining illness and effective HIV treatment were highly significant as predictors for reaching CD4 count ≤100, the CIs were very large, making the interpretation of their respective HRs less accurate. Model 3 was the most comprehensive Cox model, incorporating four main effects. While Hepatitis C status did not attain the required level of significance, this model is best at predicting the progression of HIV in patients co-infected with HIV and HCV, as reflected by the highly significant p-values for the other predictor variables. Therefore, Model 3 is the final Cox model chosen for predicting HIV disease progression to the HIV endpoint CD4 count ≤100.

6.8 Interactions

In order to determine whether there were any interactions occurring between Hepatitis C status and any of the other predictor variables in the analyses with two main effects, each interaction term was entered separately into the analyses. Of particular importance, it was hypothesized that a significant interaction might occur between age and risk behaviour. In our Cox models, risk behaviour, specifically IDU, was behaving in a protective manner (HR<1). An explanation for the protective nature of IDU behaviour is perhaps the result of the younger age of these patients. However, when this term was entered into the model, it did not achieve statistical significance, and therefore, was not included in the final Cox model. Age is regarded as an important factor when considering any Cox regression model. It is a variable that should be included in future Cox models that assess the relationship between Hepatitis C and HIV disease progression, regardless if it attains statistical significance. Of all the interaction terms, the only significant term was Hepatitis C status*AIDS-defining illness; it produced a significant improvement from the corresponding Cox model with two main effects (p-value = 0.026). In the Cox analyses with three or more main effects, no significant interaction terms were found.

6.9 Conclusions

Upon completing the analyses with a single main effect, it is important to note that there was only one variable with a significant result; the predictor variable AIDS-defining illness revealed a significant result of <0.001 and a corresponding HR of 6.3. Moreover, risk behaviour, effective treatment, baseline CD4 percent, and baseline CD4 count were the only other predictor variables to yield p-values less than 0.25; these p-values were 0.14 and 0.08, 0.24, and 0.20, respectively. Thus, instead of focusing on statistical significance, the focus was placed on HRs. Once the analyses with one main effect were carried out, analyses with two main effects were performed in which Hepatitis C status was included with one other predictor variable.

In the analyses with two main effects, there were very few significant results. The comparison of the hazard ratios in these analyses with those in the analyses with one main effect revealed that there were a small number of confounders. Risk behaviour and AIDS-defining illness are confounders for Hepatitis C status, and as would be expected Hepatitis C status was a confounder for the predictor variables risk behaviour, AIDS-defining illness, and baseline CD4 percent. Finally, there was a significant interaction between Hepatitis C status and AIDS-defining illness in the Cox model with these variables entered as the two main effects.

Finally, Cox models with three or more main effects were carried out. The model that was chosen to be most predictive of reaching the HIV endpoint of a CD4 count ≤100 included the variables Hepatitis C status, risk behaviour, effective HIV treatment, and AIDS-defining illness. It is important to note that, because the confidence intervals were fairly wide and the sample size was too small to have 80% power to detect the hazard ratios obtained in our analyses, the results should be interpreted with caution.

The Cox model is reasonably popular because it is robust. That is, even though the baseline hazard function is unspecified, reasonably good estimates of the regression coefficients, hazard ratios, and adjusted survival curves can be obtained. Results from the Cox model usually closely approximate those for the correct parametric model. Often, however, there is uncertainty surrounding which parametric model is suitable. Thus, the Cox model is considered a safe model that will give reliable results, when there is concern as to which parametric model should be chosen.

TABLE 6.1: COX REGRESSION RELATING HEPATITIS C STATUS TO EACH OF THE FOUR HIV ENDPOINTS.

HIV Endpoint	*N	β	SE (β)	Wald P-value	HR	95% CI for HR
CD4 Count ≤100	161	0.33	0.50	0.51	1.4	(0.5-3.7)
CD4 Percent ≤8%	156	0.37	0.51	0.47	1.4	(0.5-3.9)
AIDS-defining Illness	166	0.17	0.49	0.73	1.2	(0.5-3.1)
Death	177	0.47	0.88	0.60	1.6	(0.3-9.0)

*N varies due to some missing values.

TABLE 6.2: COX REGESSION WITH ONE MAIN EFFECT FOR THE HIV ENDPOINT CD4 COUNT ≤100.

Variable	Hazard Ratio	95% CI for Hazard Ratio	Wald P-value	**N
Hepatitis C Positive	1.4	0.5-3.7	0.51	160
Risk Behaviour (IDU vs. non-IDU)	0.5	0.2-1.3	**0.14**	160
Male Gender	1.0	0.2-4.7	0.97	160
Ever Jailed (Yes vs. No)	0.4	0.1-3.2	0.40	160
Baseline CD4 Count ≤500 vs. Other	2.0	0.6-6.1	**0.24**	160
Baseline CD4% ≤28% vs. Other	2.3	0.7-8.2	**0.20**	155
Aboriginal Ethnicity vs. Other	1.1	0.3-4.1	0.85	160
Caucasian Ethnicity vs. Other	0.8	0.3-2.3	0.68	160
Age ≥26 years vs. Other	2.8	0.4-22.2	0.33	160
HBsAg and/or HBsAb Positive	1.0	0.4-2.8	0.99	155
AIDS-defining illness	6.3	2.3-16.9	**<0.001**	160
No Effective HIV Treatment	2.7	0.9-8.0	**0.08**	115

***bold** type identifies p-values <0.25

**N varies due to missing values

TABLE 6.3:COX ANALYSES WITH TWO MAIN EFFECTS FOR THE HIV ENDPOINT CD4 COUNT ≤100.

Other Variables (except HCV)	Hazard Ratio		Wald P-value	
	Hep C	Other Var.	Hep C	Other Var.
Risk Behaviour (IDU vs. non-IDU)	1.9	0.4	**0.22**	**0.07**
Male Gender	1.4	1.1	0.51	0.92
Ever Jailed (Yes vs. No)	1.6	0.4	0.39	0.33
Baseline CD4 Count ≤500 vs. Other	1.5	2.1	0.43	**0.21**
Baseline CD4% ≤28% vs. Other	1.6	2.7	0.38	**0.14**
Aboriginal Ethnicity vs. Other	1.4	1.2	0.50	0.79
Caucasian Ethnicity vs. Other	1.5	0.7	0.44	0.55
Age Category ≥26years vs. Other	1.5	3.1	0.42	0.30
HBsAg and/or HBsAb Present	1.4	1.0	0.50	0.94
AIDS-defining illness	1.8	6.9	0.25	**<0.001**
No Effective HIV Treatment	1.5	2.6	0.43	**0.09**

***bold** type identifies p-values <0.25

TABLE 6.4: COX ANALYSES WITH THREE OR MORE MAIN EFFECTS FOR THE HIV ENDPOINT CD4 COUNT ≤100.

	Variables	Hazard Ratio	95% CI for Hazard Ratio	Wald P-value
Model 1 N=160	Hepatitis C Positive	2.5	(0.9-7.0)	0.09
	Risk Behaviour (IDU vs. non-IDU)	0.3	(0.1-0.8)	0.02
	AIDS-Defining Illness	8.3	(3.0-23.2)	<0.001
Model 2 N=115	Hepatitis C Positive	1.9	(0.6-5.7)	0.27
	AIDS-Defining Illness	9.9	(3.3-30.3)	<0.001
	No Effective HIV Treatment	3.3	(1.1-10.1)	0.04
Model 3 N=115	Hepatitis C Positive	2.2	(0.7-6.6)	0.16
	Risk Behaviour (IDU vs. non-IDU)	0.2	(0.0-0.7)	0.01
	AIDS-Defining Illness	23.4	(5.3-102.9)	<0.001
	No Effective HIV Treatment	7.4	(1.7-31.8)	0.01

Note: N varies due to some missing values

CHAPTER SEVEN

DISCUSSION, RECOMMENDATIONS, AND CONCLUSIONS

7.1 Discussion - Summary of Results

One of the objectives of this study was to establish the prevalence of viral Hepatitis C in a Northern Alberta cohort of HIV-infected patients. The results of this study revealed that the prevalence of Hepatitis C in this cohort of HIV positive patients is 25.6%. Congruent with the literature, our findings were that IDU was the primary risk factor associated with current HCV infection. However, considering that up to 90% of IDUs are positive for antibodies to Hepatitis C, it was somewhat surprising that only 67.4% of patients who reported a history of IDU in the whole cohort were infected with Hepatitis C. This was primarily the result of a lack of testing for Hepatitis C among 97 IDUs. However, among those IDUs tested for Hepatitis C, 87.1% were positive. On the other hand, 89.0% of patients co-infected with HIV and Hepatitis C are IDUs, 53.4% are Caucasian, 33.4% have been incarcerated at some point in their lives, and 66.9% are male.

Although of considerable interest, a smaller focus was placed on determining the interactions, if any, between Hepatitis C Virus, Hepatitis B Virus, and HIV. There were 49.7% (162/326) of Hepatitis C positive patients that were positive for Hepatitis B surface antigen or antibody. Eighty-eight percent of these Hepatitis C positive patients that are Hepatitis B surface antigen or antibody positive have a history of IDU. This can be compared to 32.3% (162/501) of Hepatitis B surface antigen positive patients who are Hepatitis C positive.

The four HIV endpoints chosen for the purposes of this study were a CD4 count ≤100, a CD4 percent ≤8%, death, and an AIDS-defining illness. The KM survival curves revealed some very interesting results. Initially, a time bias was found with the data. After correcting for this bias, a minimum of a 48-month survival time was used for the KM curves. In all of the KM survival curves relating Hepatitis C antibody status as the single main effect to each of the four HIV endpoints, patients co-infected with Hepatitis C appeared to have a worse survival probability than the group of patients infected only with HIV. However, these findings were not statistically significant.

In the Cox regression, analyses with a single main effect, two main effects, and three or more main effects were performed. In the first group of analyses with a single main effect, only Hepatitis C status was entered as a predictor variable for each of the four HIV endpoints. Hepatitis C was not found to have a statistically significant effect on any of the HIV endpoints. The measure of effect for Hepatitis C ranged from 1.2 to 1.6 for all four of the HIV endpoints. The HR was 1.2 (95% CI 0.5-3.1) for the HIV endpoint of an AIDS-defining illness, 1.4 (95% CI 0.5-3.7) for the HIV endpoint CD4 count ≤100, 1.4 (95% CI 0.5-3.9) for the HIV endpoint CD4 percent ≤8%, and 1.6 (95% CI 0.3-9.0) for the HIV endpoint death. These measures of effect are clinically important. A hazard ratio of 1.4 for the HIV endpoint of a CD4 count ≤100 can be interpreted as patients who are co-infected with Hepatitis C having a 40% increased hazard of reaching this endpoint. The fact that these hazard ratios did not attain statistical significance may be a reflection of the small sample sizes used in the analyses. However, had there been adequate sample size, a lack of statistical significance may simply indicate that there is, in fact, no statistical association between Hepatitis C and HIV disease progression.

Several other predictor variables were entered into analyses as the single main effect. The only variable to achieve significance was AIDS-defining illness; patients who have had an AIDS-defining illness have an increased hazard of 6.3 of reaching the HIV endpoint of a CD4 cell count ≤100. Effective treatment nearly attained statistical significance (p=0.08); patients who have not responded well to HIV treatment (viral load >500) have an increased hazard of 2.7 of reaching a CD4 count ≤100. Once again, the fact that the majority of the hazard ratios for the predictor variables entered into the analyses with one main effect did not attain statistical significance is likely a reflection of the small sample size.

For reasons cited in Chapter six, all analyses with two main effects were performed using the HIV endpoint CD4 count ≤100. These analyses all included Hepatitis C status and one other variable as the predictor variables for HIV disease progression to a CD4 count ≤100. It was hypothesized that if there was an association to be found between Hepatitis C and HIV disease progression, predictive factors for HIV disease progression to the HIV endpoint of a CD4 count ≤100 might include effective HIV treatment and high risk demographic factors, such as aboriginal ethnic status, IDU, and the age group ≥26 years of age.

Risk behaviour and effective HIV treatment nearly attained statistical significance as predictors for HIV disease progression when they were entered alongside Hepatitis C status in their respective models with two main effects. AIDS-defining illness remained statistically significant as a predictor variable for HIV disease progression when entered alongside Hepatitis C status. Patients who reported IDU had a decreased hazard of 0.4 of reaching a CD4 count ≤100 (p=0.07) and patients who had not responded well to HIV treatment had an increased hazard of 2.6 of reaching the HIV endpoint (p=0.09). Aboriginals had an increased hazard of 1.2 of reaching a CD4 count ≤100 when aboriginal ethnic status was entered with Hepatitis C status in the model with two main effects; however, this variable did not attain statistical significance (p=0.79). Patients ≥26 years of age had an increased hazard of 3.1 of reaching a CD4 count ≤100 in the analysis with age and Hepatitis C status as the two main effects; this finding was not statistically significant (p=0.30).

A low socioeconomic status was also hypothesized as a predictor of HIV disease progression. However, socioeconomic status was not captured in the database and thus this variable could not be evaluated. Immunologic factors such as baseline CD4 cell count and baseline CD4 percent were also hypothesized to be predictive of attaining a CD4 count ≤100. While these variables did not attain statistical significance, patients with a baseline CD4 count ≤500 and a baseline CD4 percent ≤28% had an increased hazard of attaining the HIV endpoint by factors of 2.1 (p=0.21) and 2.7 (p=0.14)), respectively.

Hepatitis C status was a confounder for the predictor variables risk behaviour, baseline CD4 percent, and AIDS-defining illness, because the crude (model with one main effect) versus adjusted (model with two main effects) hazard ratios for these predictor variables were meaningfully different. Furthermore, the predictor variables risk behaviour and AIDS-defining illness were confounders for Hepatitis C status, because the crude and adjusted hazard ratios for Hepatitis C status are meaningfully different. Please refer back to Tables 6.2 and 6.3.

7.2 Discussion - Agreement of Findings With Other Studies on HIV and HCV Co-Infection.

There are two types of studies relating to Hepatitis C and HIV co-infection, those that address the progression of HIV disease, and those that deal with HCV disease progression.

In studies that assess the progression of Hepatitis C in co-infected patients, there is a general consensus that HIV and HCV co-infection modify the natural history of chronic parentally-acquired HCV, with an unusual progression to cirrhosis HCV [37, 49]. HIV and HCV co-infection also aggravate the course of preceding long-term chronic Hepatitis C by a more marked fibrosis [14]. Moreover, it has been discovered that Hepatitis C viral load is significantly higher in HIV positive patients than in patients who are infected only with HCV [49]. It appears as though HCV replication is in some way directly influenced by the presence of HIV. While HCV-specific host immunity controls, in part, HCV replication, HCV replication also increases when the immune system is impaired by HIV [14, 36]. Paradoxically, Hepatitis C disease does not appear to improve even with effective treatment of HIV and it may, in fact, sometimes worsen.

It was the focus of this study to assess HIV disease progression in co-infected patients. Our findings were similar to those found in the literature. Table 7.1 is taken in part from a

Spanish study by Berenguer et al [66]. It is a concise presentation of the current findings to date on the natural history of HIV in patients co-infected with Hepatitis C.

Table 7.1 indicates that the results have varied and been contradictory in studies evaluating the progression of HIV in co-infected patients. Most of the studies evaluating the natural history of HIV and HCV co-infection were done before potent antiretroviral therapy was available. Earlier studies did not demonstrate either a clinical or an immunological effect of HCV and HIV co-infection on the progression of HIV. On the other hand, with one exception, more recent studies have found that co-infection with HCV has a negative impact on HIV disease progression.

A study in 1994 by Wright et al [67] found HCV did not influence the survival of HIV-infected patients with or without manifestations of AIDS. Dorrucci et al. [55] followed a cohort of 416 HIV positive patients without AIDS and from different exposure categories, of which 214 were co-infected with Hepatitis C. This study did not find any detrimental effect of co-infection with HCV. However, it was acknowledged that any such effect may have been confounded by the effect of exposure category. Moreover, there are two cross-sectional studies, which also failed to show any relationship between Hepatitis C infection and HIV disease progression [56, 57].

In the study by Ockenga et al [44], co-infection was associated with reduced survival in patients with AIDS compared to controls. In a cohort of 111 haemophiliac patients, patients infected with Hepatitis C genotype 1 experienced a significantly more rapid progression to both AIDS (p=0.009) and death (p=0.007) than did those infected with other genotypes [46]. The study published in 1998 by Piroth et al [45] followed a cohort of 238 HIV positive patients from 1993-1996, 119 of whom were Hepatitis C positive. Clinical progression was more rapid in co-infected patients. Moreover, they found the prognostic value of Hepatitis C infection to be significant for both clinical and immunological progression of HIV at early stages of HIV infection.

Haydon et al [68] performed one of the more recent studies (1998) on a cohort of IDUs; in contrast to other recent studies, this study did not find evidence that HCV infection influences the rate of progression to either clinical or immunological endpoints.

The most recent study was from the ongoing Swiss HIV Cohort Study performed by Greub et al [47], which focused on the era of potent antiretorviral therapy. They analyzed clinical progression of HIV, and the virological and immunological response to potent HAART therapy in HIV patients with or without concurrent HCV infection. Probability of progression to a new AIDS event was independently associated with HCV seropositivity (HR=1.7), and with active IDU (HR=1.4). Both of these findings were statistically significant. However, virological response to antiretroviral therapy and the probability of treatment change were not significantly associated with HCV serostatus.

After considering the inclusion and exclusion criteria, we performed univariate and bivariate Cox regression analyses on 161 patients, 111 whom were co-infected with HIV and HCV. Our findings were that HCV co-infection does not induce a significant deleterious effect on the natural history or progression of HIV disease. Our results are very similar to those of Haydon et al [68].

Similar to the findings in the above-mentioned studies, the prevalence of HCV in HIV-infected patients in our study was strongly related to the parenteral route of infection, specifically injection drug use and blood transfusion prior to the screening for HCV-antibody and HIV-antibody in the blood donor population. Thus, drug addicts and haemophiliacs often presented with the highest rates of co-infection. While there has been a large decrease in the number of haemophiliacs becoming infected with HIV or Hepatitis C owing to the screening of blood products, there is an increasing number of HIV infections among the IDU population, making the problem of co-infection for HCV and HIV more common and relevant.

It is likely that access to highly active antiretroviral drugs in recent years (since late 1996), and the resulting increase in the survival of HIV positive patients constitutes the main reason why there have been discrepancies in results between the initial and more recent studies. Other reasons for the discrepancies may be: 1) differences in study design; most of the earlier studies were cross-sectional or retrospective in nature with shorter follow-up periods, while more recent studies have been longitudinal and have evaluated cohorts of HIV positive patients from the date of HIV seroconversion, 2) differences in the type of patients enrolled and included in the study (haemophiliacs, IDUs, homosexuals), since it has been discovered that there are distinct disease trajectories among different risk groups infected with HIV, 3) very few, if any, cohorts have established the exact date for HIV seroconversion, 4) small sample sizes, and 5) failure to correct for important confounding variables such as active IDU.

7.3 Discussion - Strengths and Limitations.

This study has some valuable strengths as well as important limitations.

7.3.1 Strength - Study Design

There are strengths associated with this study design, which is a retrospective cohort study. Similar to case-control studies, retrospective cohort studies are particularly appropriate for the evaluation of diseases with long latent periods. Hepatitis C has a latent period that ranges anywhere from 20-50 years, and HIV has a latent period that ranges anywhere from 10-20 years. Furthermore, this retrospective cohort study allowed us to explore a wide range of potential etiologic factors that may relate either to HIV or HCV, as well as the interrelationships between these two factors. As such, a range of exposures were assessed among co-infected individuals and individuals infected only with HIV.

There is not a large amount of information available on the progression of either HIV disease or Hepatitis C in patients who are co-infected with HIV and HCV. This type of study design is useful for advancing the knowledge about a particular disease or outcome of interest.

Although this study was a retrospective cohort study, it is not an inception cohort, whereby all patients are identified at an early and uniform point (inception) in the course of their HIV disease. The failure to start a study of clinical course and prognosis with an inception cohort has an unpredictable effect on the results; in the majority of cases, the effect would be to make prognosis appear worse than it really is [9].

7.3.2 Limitation - Length of Follow-Up

One of the limitations of this study was the length of follow-up. Patients had been entered into the HIV database as early as 1979. Therefore, some patients have been followed for as long as 21 years. The mean and median length of follow-up for the entire database (n=1,276) were 4.6 years and 3.6 years, respectively. For patients co-infected with HCV (n=326), the mean and median length of follow-up was 3.1 years and 2.6 years, respectively. Although it may appear as though this is a sufficient amount of time for follow-up, similar studies had median lengths of follow-up that were as long as 6.8 years [68]. The reason that longer follow-up is needed is because of the nature of disease progression for HCV and HIV. Both are persistent virus infections, having significantly long disease progression periods, and thus require longer periods of follow-up to be able to detect specific outcomes or endpoints.

7.3.3 Limitation - Database Inaccuracies and Incompleteness

The main limitations of this study are a result of incompleteness and inaccuracies of the database. There are patients who may have been HIV-antibody positive, but who were never seen by an Infectious Disease physician at the University of Alberta Hospital or at one of the participating STD Clinics. This number is likely to be small (<20%), because physicians who participate in the database provide the great majority of HIV care in Northern Alberta [59]. Furthermore, the laboratory normally provides viral load testing and CD4 cell counts, and the pharmacy fills prescriptions for antiretroviral drugs only when these physicians are involved in a patient's care. The implications of this would be to underestimate the prevalence of HIV.

There are also significant issues with respect to the exact date of seropositivity for HCV, HBV, and HIV. These variables were not necessarily established at baseline. Therefore, in most instances, there are missing values, or an educated estimate is given for the date the patient was believed to have become seropositive. There are, however, some patients for whom there is information available on date last negative and date seropositive with respect to HIV. Taking the median between these two dates is the ideal for obtaining the most accurate date of HIV seroconversion. Moreover, because there were imprecise dates for HIV seroconversion in the database, a number of exclusion criteria were imposed on patients in the study; most importantly, patients must have survived a minimum of 48 months in order to be included. Therefore, patients were excluded based on their outcome, not their exposure status, as would be the case for other cohort studies. This was one of the primary limitations for the study.

Age at seroconversion is an important prognostic marker for HIV disease progression. However, there must be information on both date seropositive and date last negative, so that the midpoint between these time intervals may be calculated. Measuring age at seroconversion in this manner would be consistent with the literature. However, in this database there are many missing values with respect to date seropositive and date last negative. Thus, age at seroconversion could not reliably be incorporated into this study. Instead, age categories were created based on the date when a patient was first seen, which is a much more crude measurement.

Another limitation of the database involves the lack of information on nutritional status (BMI), health status, and alcohol consumption. Although the patients' weights were measured, there were no height measurements for the majority of patients, thus making it impossible to calculate the BMI. Alcohol consumption was not reliably documented. Augmenting the dataset to obtain these variables was not considered feasible for this study, but should be considered in future studies because of the potential relevance of measures of nutritional status to survival.

In the future, it is recommended that CD4 percent and rate of CD4 percent change be used as HIV endpoints. These have been proven to have greater prognostic value than baseline CD4 cell count. However, in our database, CD4 percent values have only been available for the past 5 years.

7.4 Recommendations and Future Research

As the HIV epidemic moves increasingly into an IDU population, co-infection is expected to become increasingly common. As HIV treatment improves, the effects of co-infection with Hepatitis viruses will increasingly determine patient outcome. It is not known what the mechanisms are whereby HCV alters HIV disease progression or, strictly speaking, if it does at all.

It is obvious that there is an urgent need for a therapeutic strategy for chronic Hepatitis C that can be made-to-order for the needs of HIV co-infected persons [26]. The outcome for HIV and Hepatitis C co-infected patients is evidently complex and will most likely change with the continued use of potent highly active antiretroviral therapy (HAART). It is known that HAART is associated with improved health outcomes for people living with HIV/AIDS. However, the full therapeutic benefit from HAART often requires near-perfect adherence to the prescribed drug regimens. Prospective studies are needed that include patients being treated for HCV infection as well. Furthermore, prospective studies are needed that will carefully assess co-infected patients receiving triple antiretroviral therapy for their HIV to more clearly determine the role that this therapy has on the outcome of co-infected individuals [24]. Because monotherapy is no longer the option of first choice for treating HCV, a combination with ribavirin must be carefully integrated into the treatment schedule in which the control of the underlying HIV remains paramount [26].

There is no current data with respect to the interactions between the respective drug regimens for HIV and HCV. Some *in vitro* evidence has suggested adverse drug interactions between NRTIs and ribavirin. The best evidence in support of an effect of Hepatitis C on HIV will be when there are better anti-HCV treatments available for co-infected patients. Ideally, these treatments would significantly increase the sustained virological response, at which point we can more clearly elucidate the effect HCV therapy would have on HIV disease. However, it is important to keep in mind that one negative effect of Hepatitis C, might be to complicate the HAART therapy for HIV due to more drug-related liver problems. This should also be more closely examined in future studies.

Using administrative databases for the purposes of epidemiologic research can be highly cost-efficient. However, it is imperative that the database be properly maintained in order that it be of use to those wishing to utilize it for research purposes. There were a number of limitations with the HIV database used for the purpose of this study. A more complete database would have included information on the following:

a) dates for HIV and HCV seroconversion
b) alcohol consumption history
c) treatment modalities for HIV and HCV
d) socioeconomic status
e) body mass index (or some measure of nutritional status)

The reasons for recommending the augmentation of the HIV database with the above information are outlined in the discussion.

7.5 Conclusions

This study advances our knowledge of the local epidemiology of HIV and Hepatitis C co-infection and helps to guide future investigations into the management of Hepatitis C in HIV-infected patients. This study demonstrates that HIV and HCV co-infection is a large and growing problem in Northern Alberta with important public health and prevention implications, as well as important implications for the prognosis and management of individual patients. While a statistically significant effect of HCV on HIV disease progression and outcome could not be demonstrated in this population, this is likely due to the limitations of a small sample size and limitations in the available data such as date of HIV seroconversion for many patients. In addition, the results may have been due to the many factors besides HCV, which also independently affect the outcome of HIV disease. Investigations must be undertaken which include basic science work that will illustrate the mechanism of any interaction between HIV and Hepatitis C. Finally, it is crucial that there be trials that consider the effect of anti-HCV therapy in HIV-infected individuals to observe whether remission of HCV has a positive impact on the course of HIV disease.

7.5.1 Conclusions - Public Health Impacts

From a public health perspective, it is apparent that HIV and HCV co-infection occurs predominantly amongst IDUs in the Northern Alberta population. In an effort to minimize the number of co-infections that will occur in the future, education and rehabilitation of IDUs is required. Efforts and interventions must be introduced, similar to those that were made by and then targeted at homosexual men in the earlier era of the HIV epidemic. Suggestions include educating IDUs about HIV and HCV, focusing specifically on the risks pertaining to the route of transmission for each infection. Other suggestions might be to introduce needle exchange programs to promote a cleaner and safer environment for those individuals who wish to continue engaging in this behaviour. In order to reduce the impact of HIV and HCV co-infection, it is clear that a combination of efforts is required.

It is crucial that patient support and education are implemented or increased for promoting adherence to HAART therapies for HIV/AIDS. One study compared a pharmacist-led intervention consisting of educational counseling and availability of follow-up telephone support with conventional dispensing of HAART pills. This intervention significantly improved adherence to HAART, and adherence to HAART significantly predicted undetectable viral load at 24 weeks [69]. Controlled trials are urgently needed to determine which interventions can significantly improve adherence to HAART, and whether these interventions that improve adherence also suppress viral load and improve clinical outcomes [69]. While it would be ideal to have patients that adhere perfectly to their drug regimens, it is not realistic. It is human nature that some patients will not strictly comply with their HAART therapy. However, by implementing patient support and education, and promoting adherence to drug regimens, it is hoped that clinical outcomes for HIV/AIDS will significantly improve.

TABLE 7.1: IMPACT OF HCV INFECTION ON THE NATURAL HISTORY OF HIV.

Author, Year	Type of Study	Population Studied	HIV and HCV Status	Association between HCV & progression to AIDS
Quan et al, 1993 [56]	Cross-sectional	224 HIV+	HIV+/HCV+ = 18 HIV+/HCV- = 206	NO
Llibre et al, 1993 [57]	Cross-sectional	92 HIV+; IDU=71; MSM=9; Heter=12	HIV+/HCV+ = 71 HIV=/HCV- = 21	NO
Wright et al, 1994 [67]	Retrospective	512 HIV+ MSM (AIDS: 224)	HIV+/HCV+ = 74 HIV+/HCV- = 438	NO
Dorucci et al, 1995 [55]	Cohort (1991-1994)	416 HIV+ without AIDS	HIV+/HCV+ =214 HIV+/HCV- = 202	NO
Ockenga et al, 1997 [44]	Cohort (1993-1994)	232 (47% with AIDS)	HIV+/HCV+ = 60 HIV+/HCV- = 172	YES
Sabin et al, 1997 [46]	Cohort (1979-1996)	111 Hemophiliacs: HIV+/HCV+	HCV genotype 1 = 70%, 2: 13%, 3: 4%, 4: 2%	YES
Piroth et al, 1998 [45]	Cohort (approx. 1993-1996)	238 HIV+	HIV+/HCV+ = 119 HIV+/HCV- = 119	YES
Haydon et al, 1998 [68]	Cohort (1985-1996)	240 HIV+ IDU	HIV+/HCV+ = 202 HIV+/HCV- = 38	NO
Greub et al, 2000 [47]	Cohort (1996-2000)	3111 HIV+ receiving HAART	HIV+/HCV+ = 1157 HIV+/HCV- = 1954	YES

Berenguer et al [66

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Appendix 1:

Conditions included in the 1993 AIDS surveillance case definition [40].

CONDITIONS INCLUDED IN THE 1993 AIDS SURVEILLANCE CASE DEFINITION [40].

Candidiasis of bronchi, trachea, or lungs
Candidiasis, esophageal
Cervical cancer, invasive
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related
Herpes simplex: chronic ulcer(s) (>1 month's duration); or bronchitis, pneumonitis, or esophagitis
Histoplasmosis, disseminated or extrapulmonary
Isoporiasis, chronic intestinal (>1 month's duration)
Kaposi's sarcoma
Lymphoma, Burkitt's (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *Mycobacterium kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis carinii pneumonia (PCP)
Pneumonnia, recurrent
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome owing to HIV

Appendix II:

Complete list of all HIV drugs.

COMPLETE LIST OF ALL HIV DRUGS

A) Non-nucleoside reverse transcriptase inhibitors (NNRTI).

Nevirapine (Viramune)
Delavirdine mesylate (Rescriptor)
Efavirenz (Sustiva)

B) Nucleoside reverse transcriptase inhibitors (NRTI).

AZT/Zidovudine/ZDV (Retrovir)
ddI/Didanosine (Videx)
ddC/Zalcitabine/Dideoxycytidine (Hivid)
Lamivudine (3TC)
ZDV + 3TC (Combivir)
d4T/Stavudine (Zerit)
Abacavir (Ziagen)

C) Protease Inhibitors (PI).

Saquinovir (Fortovase/Invirase)
Ritonavir (Norvir)
Indinavir sulfate (Crixivan)
Nelfinavir (Viracept)
Amprenavir

D) Others

Hydroxyurea (Hydrea)